Risks of Azinphos Methyl Use to the Federally Listed California Red Legged Frog
(*Rana aurora draytonii*)

Pesticide Effects Determination

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1. Executive Summary

The purpose of this assessment is to make an "effects determination" by evaluating the potential direct and indirect effects of the insecticide, azinphos methyl, on the survival, growth, and reproduction of the California red legged frog (*Rana aurora draytonii*). In addition, this assessment evaluates the potential for azinphos methyl use to result in the destruction or adverse modification of designated critical habitat for the California red legged frog (CRLF). The structure of this risk assessment is based on guidance contained in U.S. EPA's *Guidance for Ecological Risk Assessment* (U.S. EPA 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS 1998) and is consistent with procedures and methodology outlined in the Overview Document (U.S. EPA 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (USFWS/NMFS 2004).

Azinphos methyl is used throughout the United States on a limited number of agricultural commodities (almonds, Brussels sprouts, apples, low- and highbush blueberries, cherries (sweet and tart), nursery stock, parsley, pears, pistachios, and walnuts). There are no other agricultural or non-agricultural sites currently registered. Azinphos methyl is applied via airblast or ground spray application. Although the action area is likely to encompass a large area of the United States, the scope of this assessment limits consideration of the overall action area to those portions that are applicable to the protection of the CRLF and its designated critical habitat. Azinphos methyl may not be used on blueberries or parsley in the state of California. The initial area of concern for azinphos methyl is limited to those agricultural lands within the state of California where *almonds, Brussels sprouts, apples, cherries (sweet and tart), nursery stock, pears, pistachios, and walnuts* are grown. The initial area of concern represents the "footprint" of where azinphos methyl could potentially be used based on land cover information. The initial area of concern is then expanded as necessary based on the potential for direct and indirect effects above levels of concern (LOCs) which considers the fate and transport properties of the compound. The action area is defined by the land use classes designated to represent these crops in a conservative fashion and account for the fate and transport characteristics of the pesticide, including transport in streams and rivers, spray drift, and long-range transport. In general, the action area is defined as the general agricultural cropland and orchard land classes within the state of California plus those areas beyond this initial area of concern where effects above Agency levels of concern may occur. For azinphos methyl these areas beyond the intitial area of concern are defined by the distance spray drift may result in terrestrial and aquatic exposures above the LOC. Specifically, the greatest distance is defined by the risk to terrestrial organisms and is represented by a 3707 foot wide buffer. Also, an analysis was completed to assess the potential for risk to aquatic organisms due to downstream transport away from the site of application. This analysis indicates that a total of 194 kilometers of downstream extent is predicted to have exposures above the LOC. Together, the initial area of concern plus the buffered distance and the downstream extent represent the action area for azinphos methyl.

Consistent with the methodology specified in the Agency's Overview Document (U.S. EPA, 2004a), screening-level EECs, based on the PRZM/EXAMS static water body scenario, were used to derive risk quotients (RQs) for all relevant agricultural azinphos methyl uses within the action area. RQs based on screening-level EECs were used to distinguish "no effect" from "may effect" determinations for direct/indirect effects to the CRLFs and the critical habitat impact analysis.

The assessment endpoints for the CRLF included direct toxic effects on survival, reproduction, and growth of individual frogs, as well as indirect effects, such as reduction of the food source and/or modification of habitat. Risk quotients (RQs) for direct acute effects to the CRLF were calculated using acute toxicity data from the open literature for an aquatic-phase amphibian. RQS for direct chronic (reproductive, growth) effects were calculated using an estimated chronic NOAEC for amphibians based on the acute-to-chronic ratio for rainbow trout. To assess potential indirect effects to the CRLF via effects to potential prey (and consequently a reduction of available food items), toxicity data for freshwater fish and invertebrates as well as birds and mammals were considered. Aquatic and terrestrial plant toxicity data for azinphos methyl are very limited; however, available phytotoxcity studies from the open literature were considered and used qualitatively to describe potential risk to primary producers, and in turn, potential indirect effects to the CRLF.

Federally designated critical habitat has been established for the CRLF. Adverse modifications to the primary constituent elements of designated critical habitat, as defined in 50 CFR 414.12(b), were also evaluated. PCEs evaluated as part of this assessment include the following:

- Breeding aquatic habitat;
- Non-breeding aquatic habitat;
- Upland habitat; and
- Dispersal habitat.

RQs are derived as quantitative estimates of potential high-end risk. Acute and chronic RQs are compared to the Agency's Levels of Concern (LOCs) to identify instances where azinphos methyl use within the action area has the potential to adversely affect the CRLF or modify designated critical habitat. When RQs for a particular type of effect are below LOCs, the pesticide's use is considered to have "no effect" on the CRLF or its designated critical habitat. Where RQs exceed LOCs, a potential to cause adverse effects or habitat modification is identified, leading to a conclusion of "may affect". If azinphos methyl use "may affect" the CRLF, and/or cause modification to designated critical habitat, the best available information and data are considered to refine the potential for exposure and effects, and distinguish actions that are NLAA from those that are LAA. Effects determinations for direct/indirect effects to the CRLF and the critical habitat impact analysis are summarized below and presented in Tables 1.1 and 1.2.

Table 1.1 Effects Determination Summary for Direct and Indirect Effects of Azinphos Methyl on the California Red-legged Frog		
Assessment Endpoint	**Effects Determination**	**Basis**
Aquatic-Phase (Eggs, Larvae, Tadpoles, Adults)		
Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	Not Likely to Adversely Affect	Using acute amphibian toxicity data and an estimated chronic NOAEC (based on fish data), acute RQs for some of the assessed azinphos methyl uses (*i.e.*, almonds, apples, and Brussels sprouts) narrowly exceed the acute endangered species LOC of 0.05. However, if aquatic exposures are modeled assuming the management practices for 2008, the first year of the azinphos methyl phase-out, all acute RQs are below the acute listed species LOC. Chronic RQs do not exceed the LOC based on predicted exposures for 2007 or 2008.
Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	Likely to Adversely Affect	Azinphos methyl acute and chronic RQs exceed LOCs for freshwater fish and invertebrates; risk conclusions supported by field studies and adverse ecological incident reports.
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Terrestrial Phase (Juveniles and adults)		
Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	Likely to Adversely Affect	Azinphos methyl acute and chronic RQs exceed LOCs for direct effects using birds as a surrogate; risk conclusions supported by field studies and adverse ecological incident reports.
Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial vertebrates, including mammals and terrestrial phase amphibians)	Likely to Adversely Affect	Azinphos methyl acute and chronic RQs exceed LOCs for terrestrial invertebrates, mammals; risk conclusions supported by field studies and adverse ecological incident reports.
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation)	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.

Table 1.2 Effects Determination Summary for the Critical Habitat Impact Analysis		
Assessment Endpoint	**Effects Determination**	**Basis**
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.[1]	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	Habitat modificaiton	Aquatic invertebrate acute and chronic RQs exceed LOCs; field studies and incident reports support risk conclusions.
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)		
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	Habitat modificaiton	Azinphos methyl poses acute and chronic risk to prey items of the CRLF, including freshwater fish and invertebrates, small mammals, other amphibians, and terrestrial invertebrates.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Habitat modification	Azinphos methyl poses acute and chronic risk to prey items of the CRLF, including freshwater fish and invertebrates, small mammals, other amphibians, and terrestrial invertebrates. Since azinphos methyl poses acute and chronic risk to mammals, the CRLF may be affected via alteration or reduction of refugia in the form of small mammal burrows.

[1] Physico-chemical water quality parameters such as salinity, pH, and hardness are not evaluated because these processes are not biologically mediated and, therefore, are not relevant to the endpoints included in this assessment.

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (i.e., food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (i.e., attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available. Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of CRLF life stages within specific recovery units and/or designated critical habitat within the action area. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the species.
- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual frogs and potential adverse modification to critical habitat.

2. Problem Formulation

Problem formulation provides a strategic framework for the risk assessment. By identifying the important components of the problem, it focuses the assessment on the most relevant life history stages, habitat components, chemical properties, exposure routes, and endpoints. The structure of this risk assessment is based on guidance contained in U.S. EPA's *Guidance for Ecological Risk Assessment* (U.S. EPA 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS 1998) and is consistent with procedures and methodology outlined in the Overview Document (U.S. EPA 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (USFWS/NMFS 2004).

2.1 Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the federally threatened California red-legged frog *(Rana aurora draytonii*) (CRLF) arising from FIFRA regulatory actions regarding use of azinphos methyl on almonds, Brussels sprouts, apples, low- and highbush blueberries, cherries (sweet and tart), nursery stock, parsley, pears, pistachios, and walnuts. A total of 35 additional uses were either cancelled immediately at the time of the IRED in 2001 or were phased out over a 4-year period between 2001 and 2005. Those uses are no longer registered and are not part of this federal action. In addition, this assessment evaluates whether these actions can be expected to result in the destruction or adverse modification of the species' critical habitat. Key biological information for the CRLF is included in Section 2.5, and designated critical habitat information for the species is provided in Section 2.6 of this assessment. This ecological risk assessment has been prepared as part of the *Center for Biological Diversity (CBD) vs. EPA et al*. (Case No. 02-1580-JSW(JL)) settlement entered in the Federal District Court for the Northern District of California on October 20, 2006. It is one in a series of endangered species effects determinations for pesticide active ingredients involved in this litigation.

In this endangered species assessment, direct and indirect effects to the CRLF and potential adverse modification to its critical habitat are evaluated in accordance with the methods (both screening level and species-specific refinements, when appropriate) described in the Agency's Overview Document (U.S. EPA 2004a).

In accordance with the Overview Document, provisions of the ESA, and the Services' *Endangered Species Consultation Handbook*, the assessment of effects associated with registrations of azinphos methyl are based on an action area. The action area is considered to be the area directly or indirectly affected by the federal action, as indicated by the exceedance of Agency Levels of Concern (LOCs) used to evaluate direct or indirect effects. It is acknowledged that the action area for a national-level FIFRA regulatory decision associated with a use of azinphos methyl may potentially involve numerous areas throughout the United States and its Territories. However, for the purposes of this assessment, attention will be focused on relevant sections of the action area including those geographic areas associated with locations of the CRLF and its designated critical habitat within the state of California.

As part of the "effects determination," one of the following three conclusions will be reached regarding the potential for registration of azinphos methyl at the use sites described in this document to affect CRLF individuals and/or result in the destruction or adverse modification of designated CRLF critical habitat:

- "No effect";
- "May affect, but not likely to adversely affect"; or
- "May affect and likely to adversely affect".

Critical habitat identifies specific areas that have the physical and biological features, (known as primary constituent elements or PCEs) essential to the conservation of listed species. The PCEs for CRLFs are aquatic and upland areas where suitable breeding and non-breeding aquatic habitat is located, interspersed with upland foraging and dispersal habitat (Section 2.6).

If the results of initial screening-level assessment methods show no direct or indirect effects (no LOC exceedances) upon individual CRLFs or upon the PCEs of the species' designated critical habitat, a "no effect" determination is made for the FIFRA regulatory action regarding azinphos methyl as it relates to this species and its designated critical habitat. If, however, direct or indirect effects to individual CRLFs are anticipated and/or effects may impact the PCEs of the CRLF's designated critical habitat, a preliminary "may affect" determination is made for the FIFRA regulatory action regarding azinphos methyl.

If a determination is made that use of azinphos methyl within the action area(s) associated with the CRLF "may affect" this species and/or its designated critical habitat, additional information is considered to refine the potential for exposure and for effects to the CRLF and other taxonomic groups upon which these species depend (e.g.., aquatic and terrestrial vertebrates and invertebrates, aquatic plants, riparian vegetation, etc.). Additional information, including spatial analysis (to determine the overlay of CRLF habitat with azinphos methyl use) and further evaluation of the potential impact of azinphos methyl on the PCEs is also used to determine whether destruction or adverse modification to designated critical habitat may occur. Based on the refined information, the Agency uses the best available information to distinguish those actions that "may affect, but are not likely to adversely affect" from those actions that "may affect and are likely to adversely affect" the CRLF and/or the PCEs of its designated critical habitat. This information is presented as part of the Risk Characterization in Section 5 of this document.

The Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitat. Because azinphos methyl is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for azinphos methyl is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked to biologically mediated processes (i.e., the biological resource requirements for the listed species associated with the critical habitat or important physical aspects of the habitat that may be reasonably influenced through biological processes). Activities that may destroy or adversely modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to use of azinphos methyl that may alter the PCEs of the CRLF's critical habitat form the basis of the critical habitat impact analysis. Actions that may affect the CRLF's designated critical habitat and jeopardize the continued existence of the species have been identified by the Services and are discussed further in Section 2.6.

2.2 Scope

Azinphos methyl is currently registered for ten crop uses, making it geographically restricted to several high use locations, including the Shenandoah and Cumberland Valleys, central Washington, Central Valley of California, and Michigan. These uses are almonds, apples, blueberries (low- and highbush), Brussels sprouts, cherries (sweet and tart), nursery stock, parsley, pears, pistachios, and walnuts. For the purposes of this assessment, only the uses in California are expected to result in potential exposures to the CRLF; therefore, uses outside this geographic range, including blueberries and parsley, are not considered. More detail on the range and limitation of these uses may be found in the 2005 follow-up to the reregistration eligibility decision (RED) risk assessment (DP barcode D307568, dated September 29, 2005).

The end result of the EPA pesticide registration process (*i.e.*, the FIFRA regulatory action) is an approved product label. The label is a legal document that stipulates how and where a given pesticide may be used. Product labels (also known as end-use labels) describe the formulation type (*e.g.*, liquid or granular), acceptable methods of application, approved use sites, and any restrictions on how applications may be conducted. Thus, the use or potential use of azinphos methyl in accordance with the approved product labels for California is “the action” relevant to this ecological risk assessment.

This ecological risk assessment is for currently registered uses of azinphos methyl in portions of the action area that are reasonably assumed to be biologically relevant to the CRLF and its designated critical habitat. Further discussion of the action area for the CRLF and its critical habitat is provided in Section 2.7.

Degradates of azinphos methyl include anthranilic acid, methyl anthranilate, azinphos methyl oxygen analog, mercaptomethyl benzazimide, hydroxymethyl benzazimide, benzazamide, and *bis*-methyl benzazamide sulfide, and methyl benzazimide sulfonic acid. Because of the limited concentrations of the identified degradates and their properties, this risk assessment has been based solely on the parent.

Azinphos methyl is an organophosphate (OP) insecticide and many of the OPs have been documented to form oxons as a degradation product. The oxons tend to be significantly more toxic than the parent compound. The formation of oxon is dependent on oxidative desulfonation (cleavage of P=S bond to form P=O bond). This transformation can occur through photooxidation, chemical oxidation in the presence of dissolved O_2 in water, oxidixizing agents such chlorine or potassium permanganate, and enzyme mediated oxidation from oxidases (Tiernery et al., 2001). The azinphos methyl oxon has been documented to form via chlorination and photolytic oxidation. As with the other degradates described above there is a lack of data on the environmental fate, toxicity, and occurrence in the environment, and thus, the oxon of azinphos methyl has not been quantitatively evaluated in this assessment. However, the potential impact of occurrence of azinphos methyl oxon has been qualitatively characterized.

This assessment only considers the potential effects of azinphos methyl insecticide exposure to the CRLF. The Agency does not routinely include, in its risk assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively in accordance with the Agency's Overview Document and the Services' Evaluation Memorandum (U.S., EPA 2004; USFWS/NMFS 2004). Azinphos methyl does not have any registered products that contain multiple active ingredients.

Sublethal effects, such as cholinesterase inhibition and behavior alteration, are discussed qualitatively in this assessment since it is not possible to quantitatively link effects such as these to the selected assessment endpoints for the CRLF (*i.e.*, survival, growth, and reproduction of individuals and maintenance of critical habitat PCEs). Further detail on sublethal effects of azinphos methyl is provided in Sections 4.1.1.4 and 4.2.1.3, for fish and birds, respectively.

2.3 Previous Assessments

In 1999, the Agency assessed the potential ecological risks associated with the use of azinphos methyl on a variety of agricultural uses. Mitigation efforts following the 2001 IRED resulted in the cancellation (with a phase-out period) of several uses and time-limited re-registration of several other uses. In 2005, the Agency reassessed the ecological risks associated with the remaining uses of azinphos methyl on almonds, Brussels sprouts, apples, low- and highbush blueberries, cherries (sweet and tart), nursery stock, parsley, pears, pistachios, and walnuts, taking into account the label specifications at the time, including application rates, methods, and mandatory buffer strips (DP barcode D307568, dated September 29, 2005). Risk conclusions from that assessment indicated acute and chronic risks to aquatic animals (*i.e.*, fish, aquatic-phase amphibians, invertebrates) and terrestrial animals (*i.e.*, birds, terrestrial-phase amphibians, mammals). Based on that evaluation of the risks and benefits of the 10 remaining uses of azinphos methyl, the Agency concluded, in 2006, that these uses will be phased out by 2012. The use of azinphos methyl on Brussels sprouts and nursery stock will be phased out in 2007; use on almonds, walnuts, and pistachios will be phased out in 2009; and use on apples/crabapples, blueberries, cherries, pears and parsley will be phased out in 2012.

On July 31, 2003, EPA initiated formal consultation relative to potential risks of azinphos methyl use to 25 Environmentally Significant Units (ESUs) of Pacific salmon and steelhead. EPA's assessment resulted in a determination that azinphos methyl use was Likely to Adverely Affect 25 ESU's based on its acute toxicity to fish, the potential for indirect effects due to acute and chronic risks to aquatic invertebrate food supply, and based on known or potential use of azinphos methyl on crops within habitats and migration corridors of each of the 25 ESUs. The assessment supporting that determination included uses of azinphos methyl that are no longer registered.

2.4 Stressor Source and Distribution

2.4.1 Environmental Fate Assessment

Azinphos methyl is mobile (K_f = 12-27) and can reach surface water dissolved in runoff, but it is not likely to leach to ground water in most situations. It is moderately persistent with aerobic soil metabolism DT_{50} (time to dissipate 50% of the compound) of 27 d. Azinphos methyl degrades rapidly by direct aqueous photolysis ($T_{1/2}$ = 77 h), but rather slowly by soil photolysis ($T_{1/2}$ = 180 d). Hydrolysis is alkaline catalyzed and is fairly rapid at high pH, on the order of several days. It is moderately persistent at acid and neutral pH. There is some uncertainty in the assessment of the hydrolysis data because data were not collected below 30° C. There are data on the degradates formed through aerobic aquatic metabolism, but no usable rate data are available.

Degradates include anthranilic acid, methyl anthranilate, azinphos methyl oxygen analog, mercaptomethyl benzazimide, hydroxymethyl benzazimide, benzazamide, *bis*-methyl benzazamide sulfide, and methyl benzazimide sulfonic acid. Because of the limited concentrations of the identified degradates and their properties, this risk assessment has been based solely on the parent. To the extent that toxic degradates were present but not considered, the risk is commensurately increased. However, this is not a major limitation of this assessment, since levels of concern are exceeded by the parent alone. Furthermore, none of the degradates that are produced by metabolic pathways, which are the primary routes of degradation for azinphos methyl, are present at any time at concentrations greater than 10% of the nominal starting concentration of the parent, so they would not be expected to contribute substantially to the total toxicity of azinphos methyl in the environment.

Azinphos methyl oxon has been documented forming at up to 5% of applied parent amount in selected environmental fate studies. Specifically, azinphos methyl oxon was determined to form at a maximum of 5% of applied at 190 days under aerobic conditions and was detected at 3% of applied at 240 hours (10 days) due to photodegradation. There are no specific environmental fate studies for azinphos methyl oxon and there is insufficient information in these studies to estimate the formation and decline of azinphos methyl oxon under these conditions (see Section 3.2.7 for more details).

A second source of uncertainty in the fate assessment is due to the field dissipation studies. The two guideline studies are both from California and are of limited quality due to very poor recoveries at initiation of the study. In addition, these studies were run on fairly alkaline soils (pH = 6.9 - 8.7), so they represent locations where azinphos methyl is somewhat less persistent. Two non-guideline studies from Georgia and Mississippi suggest that DT_{50}s in the Southeast may be relatively short, at 3 and 8 days, respectively. However, these studies only sampled the top inch of soil.

In general, contamination of groundwater is not considered to be a concern for azinphos methyl. Azinphos methyl is only moderately persistent and degrades rapidly by hydrolysis; therefore, it is not expected to reach groundwater. A review of available monitoring data from the USGS NAWQA program for azinphos methyl in groundwater indicates that of a total of 675 samples collected in California between 1993 and 2005; only one sample had a positive detection at an estimated concentration of 0.0139 ppb.

In general, the laboratory fate data for parent azinphos methyl provides a reasonable level of confidence for the risk assessment. In contrast to most other pesticides, there is a fair amount (7 half-life values) of foliar dissipation data. Additional metabolism data would increase our confidence in the chronic exposure assessment and may result in reduced estimated environmental concentrations (EECs).

A more complete summary of the environmental fate properties of azinphos methyl may be found in the Ecological Risk Assessment for azinphos methyl conducted during completion of the reregistration eligibility decision (RED) dated September 29, 2005 (DP Barcode D307568).

2.4.2 Environmental Transport Assessment

Potential transport mechanisms include pesticide surface water runoff, spray drift, and secondary drift of volatilized or soil-bound residues leading to deposition onto nearby or more distant ecosystems. The magnitude of pesticide transport via secondary drift depends on the pesticide's ability to be mobilized into air and its eventual removal through wet and dry deposition of gases/particles and photochemical reactions in the atmosphere. A number of studies have documented atmospheric transport and redeposition of pesticides generically from the Central Valley to the Sierra Nevada mountains (Fellers et al., 2004, Sparling et al., 2001, LeNoir et al., 1999, and McConnell et al., 1998). Prevailing winds blow across the Central Valley eastward to the Sierra Nevada mountains, transporting airborne industrial and agricultural pollutants into Sierra Nevada ecosystems (Fellers et al., 2004, LeNoir et al., 1999, and McConnell et al., 1998). Therefore, physicochemical properties of the pesticide that describe its potential to enter the air from water or soil (e.g., Henry's Law constant and vapor pressure), pesticide use, modeled estimated concentrations in water and air, and available air monitoring data from the Central Valley where azinphos methyl has been detected and the Sierra Nevadas (azinphos methyl has not been detected in any air sampling from the Sierra Nevada mountains) are considered in evaluating the potential for atmospheric transport of azinphos methyl to habitat for the CRLF.

In general, deposition of drifting or volatilized pesticides is expected to be greatest close to the site of application. Computer models of spray drift (AgDRIFT or AGDISP) are used to determine if the exposures to aquatic and terrestrial organisms are below the Agency's Levels of Concern (LOCs). If the limit of exposure that is below the LOC can be determined using AgDRIFT or AGDISP, longer-range transport is not considered in defining the action area. For example, if a buffer zone <1,000 feet (the optimal range for AgDRIFT and AGDISP models) results in terrestrial and aquatic exposures that are below LOCs, no further drift analysis is required. If exposures exceeding LOCs are expected beyond the standard modeling range of AgDRIFT or AGDISP, the Gaussian extension feature of AGDISP may be used. In addition to the use of spray drift models to determine potential off-site transport of pesticides, other factors such as available air monitoring data and the physicochemical properties of the chemical are also considered.

For azinphos methyl, the principal routes of transport from the application site are expected to be runoff and spray drift due to its mobility and moderate persistence. Azinphos methyl has also been documented to occur in air monitoring samples; however, these exposures appear to be related to spray drift and not indicative of long-range transport away from the area of application. In a study conducted in 1987 in Kern county (Seiber, et al, 19897), samples were collected between 100 meters and ¼ mile from the site of application and analyzed for azinphos methyl. In a study conducted in 1994 in Glenn county (Fitzell, et al, 1994), samples were collected adjacent to a treated field and analyzed for azinphos methyl. In both cases, azinphos methyl concentrations were in the part per trillion (ppt) range. Typically, air monitoring studies do not distinguish the route of transport associated with the detections. The location of the available air monitoring for azinphos methyl in Glenn and Kern counties suggest that these detections are related to nearby sources and are more likely due to spray drift than long-range transport. Also, the vapor pressure of azinphos methyl (2.2×10^{-7} torr) suggests that transport beyond that associated with spray drift is not likely.

2.4.3 Mechanism of Action

Azinphos methyl is an organophosphate insecticide, and toxicity is elicited via inhibition of the enzyme acetylcholinesterase, which cleaves the neurotransmitter acetylcholine. Inhibition of acetylcholinesterase interferes with proper neurotransmission in cholinergic synapses and neuromuscular junctions.

2.4.4 Use Characterization

There are 10 remaining uses of azinphos methyl, which will be phased out by 2012. The use of azinphos methyl on Brussels sprouts and nursery stock will be phased out in 2007; use on almonds, walnuts, and pistachios will be phased out in 2009; and use on apples/crabapples, blueberries, cherries, pears and parsley will be phased out in 2012. All of these uses are relevant to California except blueberries and parsley, which have geographically restricted uses (according to the label) and may not be used in California. During the azinphos methyl phase-out, several risk mitigation measures were implemented, including a mandatory reduction of annual application rates. **Table 2.1** presents the application rates and management practices relevant to 2007 while **Table 2.2** summarizes the risk mitigation scheme for the azinphos methyl phase-out. Environmental exposures will be estimated for the assessed uses according to the label for 2007 in order to be conservative. In the risk description (**Section 5.2**), there is additional analysis based on the management practices for 2008, the first year of the phase-out.

Table 2.1. Azinphos methyl application rates and management practices for **2007**.

Crop	Max. Rate (lbs a.i./A)	Max. No. Apps.	Minimum Interval (days)	Buffer Width (ft)	Method
Almonds[1]	2	1	NA	25	air blast
Apples[1]	1.5	32	7 d	25	air blast
Brussels sprouts	0.75	1	NA	25	ground spray
Cherries[1,3]	0.75	2	14 d	25	air blast
Nursery Stock4	1	4	10 d	25	air blast
Pears[1]	1.5	2	7 d	25	air blast
Pistachios[1]	2	1	NA	25	air blast
Walnuts[1]	2	1	NA	25	air blast

[1] No dormant application allowed
[2] Last application of 1.0 lb $acre^{-1}$ as yearly maximum is 4 lb $acre^{-1}$.
[3] Several azinphos methyl products are restricted from application to cherries before harvest in California
[4]The ornamental use specifically excludes Christmas trees.

Table 2.2. Azinphos methyl application rates and management practices for the duration of the phase-out.

Crop	Year				
	2008	2009	2010	2011	2012
Almonds	Yearly Max: 2 lbs a.i./A, 1 app. Only apply June – August. Buffer: 300 ft in Butte, Colusa, Glenn, Madera, Merced, San Joaquin, Solano, Stanislaus, Sutter, Tehama, Yolo, and Yuba counties; 500 ft in all other CA counties		Use no longer permitted.		
Apples	Yearly Max: 3 lbs a.i./A Single Max: 1.5 lbs a.i./A Minimum App. Interval: 7 d Buffer: 60 feet		Yearly Max: 2 lbs a.i./A Single Max: 1.5 lbs a.i./A Minimum App. Interval: 7 d Buffer: 60 feet	Yearly Max: 1.5 lbs a.i./A Single Max: 1.5 lbs a.i./A Minimum App. Interval: 7 d Buffer: 60 feet	
Brussels Sprouts	Use no longer permitted.				
Cherries (Sweet and Tart)	Yearly Max: 1.5 lbs a.i./A Single Max: 0.75 lbs a.i./A Apply after fruit harvest and before leaf fall. Minimum App. Interval: 14 d Buffer: 60 feet		Yearly Max: 0.75 lbs a.i./A Single Max: 0.75 lbs a.i./A Apply after fruit harvest and before leaf fall. Minimum App. Interval: 14 d Buffer: 60 feet		
Nursery Stock	Use no longer permitted.				
Pears	Yearly Max: 3 lbs a.i./A Single Max: 1.5 lbs a.i./A Minimum App. Interval: 7 d Buffer: 60 feet		Yearly Max: 2 lbs a.i./A Single Max: 1.5 lbs a.i./A Minimum App. Interval: 7 d Buffer: 60 feet	Yearly Max: 1.5 lbs a.i./A Single Max: 1.5 lbs a.i./A Minimum App. Interval: 7 d Buffer: 60 feet	
Pistachios	Yearly Max: 2 lbs a.i./A, 1 app. Only apply June – October Buffer: 500 ft in all CA counties		Use no longer permitted.		
Walnuts	Yearly Max: 2 lbs a.i./A, 1 app. Only apply June – August Buffer: 500 ft in all CA counties		Use no longer permitted.		

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information (Kaul and Jones, 2006) using state-level usage data obtained from USDA-NASS[2], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature), and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[3]. CDPR PUR is considered a more comprehensive source of usage data than USDA-NASS or EPA proprietary databases, and thus the usage data reported for azinphos methyl by county in this California-specific assessment were generated using CDPR PUR data. Five years (2002-2005) of usage data were included in this analysis. Data from CDPR PUR

[2] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.
[3] The California Department of Pesticide Regulation's Pesticide Use Reporting database provides a census of pesticide applications in the state. See http://www.cdpr.ca.gov/docs/pur/purmain.htm.

were obtained for every pesticide application made on every use site at the section level (approximately one square mile) of the public land survey system. BEAD summarized these data to the county level by site, pesticide, and unit treated. Calculating county-level usage involved summarizing across all applications made within a section and then across all sections within a county for each use site and for each pesticide. The county level usage data that were calculated include: average annual pounds applied, average annual area treated, and average and maximum application rate across all five years. The units of area treated are also provided where available. Average pounds applied and average acres treated were calculated by averaging the four years of total pounds and total area treated for each chemical-county-site-unit treated and included years with zero reported usage where present. Similarly, the application rates reported as an average, 95th percentile, 99th percentile, and maximum represent the average across all four years but does not include years with no reported use. For example, the average application rate is calculated by first calculating an annual average for each chemical-county-use-site and then averaging this value across the four years worth of averages. Similarly, the average maximum application rate represents the four year average of all maximum reported values by chemical-county-use-site. More detail on this estimation is provided in the memorandum from BEAD dated May 17, 2007 (Kaul et al, 2007).

Between 2002 and 2005 azinphos methyl was reportedly used in 37 counties in California. The principal use was on orchard and vineyard crops including almonds, apples, apricots, cherries, citrus, grapes, lemon, nectarines, orange, peach, pear, pistachio, plum, prune, quince, and walnuts. Non-orchard uses included blackberry, bok choy, broccoli, brussel sprouts, cabbage, cauliflower, celery, Chinese cabbage, cotton, garlic, onion, raspberry, potato, tomato, and strawberry. Most of these non orchard/vineyard applications were limited to three or fewer counties. In addition, non-agricultural applications were reported as landscape maintenance, greenhouse flowers, structural pest control as well as several applications as research commodities (also limited to a few counties for each use). The uses considered in this risk assessment represent all currently registered uses according to a review of all current labels. No other uses are relevant to this assessment. Any reported use, such as may be seen in the CDPR PUR database, represent either historic uses that have been cancelled, mis-reported uses, or mis-use. Historical uses, mis-reported uses, and misuse are not considered part of the federal action and, therefore, are not considered in this assessment.

The greatest average usage (average of pounds applied per commodity across all four years) was to almonds in Kern county at 24,784 lbs. By far, the greatest usage of azinphos methyl in California is to almonds at an average of approximately 48,000 lbs annually, followed by pistachios at approximately 29,000 lbs annually, apples at an approximate average of 18,000 lbs annually, pears at 11,000 lbs annually, and walnuts at 7,000 lbs annually. All remaining crops had less than 1000 lbs applied annually, and one use (nursery stock) had one reported application of 2 lbs in Santa Clara County. A summary of azinphos methyl usage for all California use sites is provided below in **Table 2.3.**

Table 2.3 Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) from 2002 to 2005 for currently registered azinphos methyl uses

Crop	Total Annual Applied (lbs)	sum of average area treated (acres)	average of average rate (lb a.i./A)	average of 95% application rate (lb a.i./A)	average of 99% application rate (lb a.i./A)
almond	48,320	1692.9	1.8	2.2	8.5
apple	17,808	507.5	1.5	1.6	2.3
brussel sprouts	409	263.0	0.8	4.1	5.8
cherries	43	11.3	0.8	0.8	0.8
nursery stock	0.5	0.5	1.0	1.0	1.0
pistachio	28,868	2252.1	2.3	2.8	6.1
pears	10,920	389.0	1.5	3.2	4.1
walnuts	6,768	209.7	5.1	6.2	6.2

2.5 Assessed Species

The CRLF was federally listed as a threatened species by USFWS effective June 24, 1996 (USFWS 1996). It is one of two subspecies of the red-legged frog and is the largest native frog in the western United States (USFWS 2002). A brief summary of information regarding CRLF distribution, reproduction, diet, and habitat requirements is provided in Sections 2.5.1 through 2.5.4, respectively. Further information on the status, distribution, and life history of and specific threats to the CRLF is provided in **Attachment 1.**

Final critical habitat for the CRLF was designated by USFWS on April 13, 2006 (USFWS 2006; 71 FR 19244-19346). Further information on designated critical habitat for the CRLF is provided in Section 2.6.

2.5.1 Distribution

The CRLF is endemic to California and Baja California (Mexico) and historically inhabited 46 counties in California including the Central Valley and both coastal and interior mountain ranges (USFWS 1996). Its range has been reduced by about 70%, and the species currently resides in 22 counties in California (USFWS 1996). The species has an elevation range of near sea level to 1,500 meters (5,200 feet) (Jennings and Hayes 1994); however, nearly all of the known CRLF populations have been documented below 1,050 meters (3,500 feet) (USFWS 2002).

Populations currently exist along the northern California coast, northern Transverse Ranges (USFWS 2002), foothills of the Sierra Nevada (5-6 populations), and in southern California south of Santa Barbara (two populations) (Fellers 2005a). Relatively larger numbers of CRLFs are located between Marin and Santa Barbara Counties (Jennings and Hayes 1994). A total of 243 streams or drainages are believed to be currently occupied by the species, with the greatest numbers in Monterey, San Luis Obispo, and Santa Barbara counties (USFWS 1996). Occupied drainages or watersheds include all bodies of water that support CRLFs (*i.e.*, streams, creeks, tributaries, associated natural and artificial ponds, and adjacent drainages), and habitats through which CRLFs can move (i.e., riparian vegetation, uplands) (USFWS 2002).

The distribution of CRLFs within California is addressed in this assessment using four categories of location including recovery units, core areas, designated critical habitat, and known occurrences of the CRLF reported in the California Natural Diversity Database (CNDDB) that are not included within core areas and/or designated critical habitat (see Figure 2.a); recovery units, core areas, and other known occurrences of the CRLF from the CNDDB are described in further detail in this section, and designated critical habitat is addressed in Section 2.6. Recovery units are large areas defined at the watershed level that have similar conservation needs and management strategies. The recovery unit is primarily an administrative designation, and land area within the recovery unit boundary is not exclusively CRLF habitat. Core areas are smaller areas within the recovery units that comprise portions of the species' historic and current range and have been determined by USFWS to be important in the preservation of the species. Designated critical habitat is generally contained within the core areas, although a number of critical habitat units are outside the boundaries of core areas, but within the boundaries of the recovery units. Additional information on CRLF occurrences from the CNDDB is used to cover the current range of the species not included in core areas and/or designated critical habitat, but within the recovery units.

Recovery Units

Eight recovery units have been established by USFWS for the CRLF. These areas are considered essential to the recovery of the species, and the status of the CRLF "may be considered within the smaller scale of the recovery units, as opposed to the statewide range" (USFWS 2002). Recovery units reflect areas with similar conservation needs and population statuses, and therefore, similar recovery goals. The eight units described for the CRLF are delineated by watershed boundaries defined by US Geological Survey hydrologic units and are limited to the elevation maximum for the species of 1,500 m above sea level. The eight recovery units for the CRLF are listed in **Table 2.4** and shown in **Figure 2.a.**

Core Areas

USFWS has designated 35 core areas across the eight recovery units to focus their recovery efforts for the CRLF (see Figure 2.a). **Table 2.4** summarizes the geographical relationship among recovery units, core areas, and designated critical habitat. The core areas, which are distributed throughout portions of the historic and current range of the species, represent areas that allow for long-term viability of existing populations and reestablishment of populations within historic range. These areas were selected because they: 1) contain existing viable populations; or 2) they contribute to the connectivity of other habitat areas (USFWS 2002). Core area protection and enhancement are vital for maintenance and expansion of the CRLF's distribution and population throughout its range.

For purposes of this assessment, designated critical habitat, currently occupied (post-1985) core areas, and additional known occurrences of the CRLF from the CNDDB are considered. Each type of location information is evaluated within the broader context of recovery units. For example, if no labeled uses of azinphos methyl occur (or if labeled uses occur at predicted exposures less than the Agency's LOCs) within an entire recovery unit, that particular recovery unit would not be included in the action area and a "no effect" determination would be made for all designated critical habitat, currently occupied core areas, and other known CNDDB occurrences within that recovery unit. Historically occupied sections of the core areas are not evaluated as part of this assessment because the USFWS Recovery Plan (USFWS 2002) indicates that CRLFs are extirpated from these areas. A summary of currently and historically occupied core areas is provided in **Table 2.4** (currently occupied core areas are bolded). While core areas are considered essential for recovery of the CRLF, core areas are not federally-designated critical habitat, although designated critical habitat is generally contained within these core recovery areas. It should be noted, however, that several critical habitat units are located outside of the core areas, but within the recovery units. The focus of this assessment is currently occupied core areas, designated critical habitat, and other known CNDDB CRLF occurrences within the recovery units. Federally-designated critical habitat for the CRLF is further explained in Section 2.6.

Table 2.4. California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat

Recovery Unit [1] (Figure 2.a)	**Core Areas [2,7] (Figure 2.a)**	**Critical Habitat Units [3]**	**Currently Occupied (post-1985) [4]**	**Historically Occupied [4]**
Sierra Nevada Foothills and Central Valley (1) (eastern boundary is the 1,500m elevation line)	**Feather River (1)**	BUT-1A-B	✓	
	Yuba River-S. Fork Feather River (2)	YUB-1		
	--	NEV-1	✓[6]	
	Traverse Creek/Middle Fork American River/Rubicon (3)	--	✓	
	Consumnes River (4)	ELD-1	✓	
	S. Fork Calaveras River (5)	--		✓
	Tuolumne River (6)	--		✓
	Piney Creek (7)	--		✓
	East San Francisco Bay (partial)(16)	--	✓	
North Coast Range Foothills and Western Sacramento River Valley (2)	**Cottonwood Creek (8)**	--	✓	
	Putah Creek-Cache Creek (9)	--		✓
North Coast and North San Francisco Bay (3)	Putah Creek-Cache Creek (partial) (9)	--		✓
	Lake Berryessa Tributaries (10)	NAP-1	✓	
	Upper Sonoma Creek (11)	--	✓	
	Petaluma Creek-Sonoma Creek (12)	--	✓	
	Pt. Reyes Peninsula (13)	MRN-1, MRN-2	✓	
	Belvedere Lagoon (14)	--	✓	
	Jameson Canyon-Lower Napa River (15)	SOL-1	✓	
South and East San Francisco Bay (4)	--	CCS-1A	✓[6]	
	East San Francisco Bay (partial) (16)	ALA-1A, ALA-1B, STC-1B	✓	
	--	STC-1A	✓[6]	
	South San Francisco Bay (partial) (18)	SNM-1A	✓	
Central Coast (5)	**South San Francisco Bay (partial) (18)**	SNM-1A, SNM-2C, SCZ-1	✓	
	Watsonville Slough- Elkhorn Slough (partial) (19)	SCZ-2 [5], MNT-1 [5]	✓	
	Carmel River-Santa Lucia (20)	MNT-2	✓	

Table 2.4. California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat				
Recovery Unit [1] (Figure 2.a)	**Core Areas [2,7] (Figure 2.a)**	**Critical Habitat Units [3]**	**Currently Occupied (post-1985) [4]**	**Historically Occupied [4]**
Diablo Range and Salinas Valley (6)	**Estero Bay (22)**	--	✓	
	Arroyo Grande Creek (23)	SLO-8	✓	
	Santa Maria River-Santa Ynez River (24)	--	✓	
	East San Francisco Bay (partial) (16)	MER-1A-B	✓	
	--	SNB-1, SBB-2	✓[6]	
	Santa Clara Valley (17)	--	✓	
	Watsonville Slough- Elkhorn Slough (partial)(19)	--	✓	
	Carmel River-Santa Lucia (partial)(20)	--	✓	
	Gablan Range (21)	SNB-3	✓	
	Estrella River (28)	SLO-1	✓	
	--	SLO-8	✓[6]	
Northern Transverse Ranges and Tehachapi Mountains (7)	**Santa Maria River-Santa Ynez River (24)**	STB-4, STB-5, STB-7	✓	
	Sisquoc River (25)	STB-1, STB-3	✓	
	Ventura River-Santa Clara River (26)	VEN-1, VEN-2, VEN-3	✓	
	--	LOS-1	✓[6]	
Southern Transverse and Peninsular Ranges (8)	**Santa Monica Bay-Ventura Coastal Streams (27)**	--	✓	
	San Gabriel Mountain (29)	--		✓
	Forks of the Mojave (30)	--		✓
	Santa Ana Mountain (31)	--		✓
	Santa Rosa Plateau (32)	--	✓	
	San Luis Rey (33)	--		✓
	Sweetwater (34)	--		✓
	Laguna Mountain (35)	--		✓

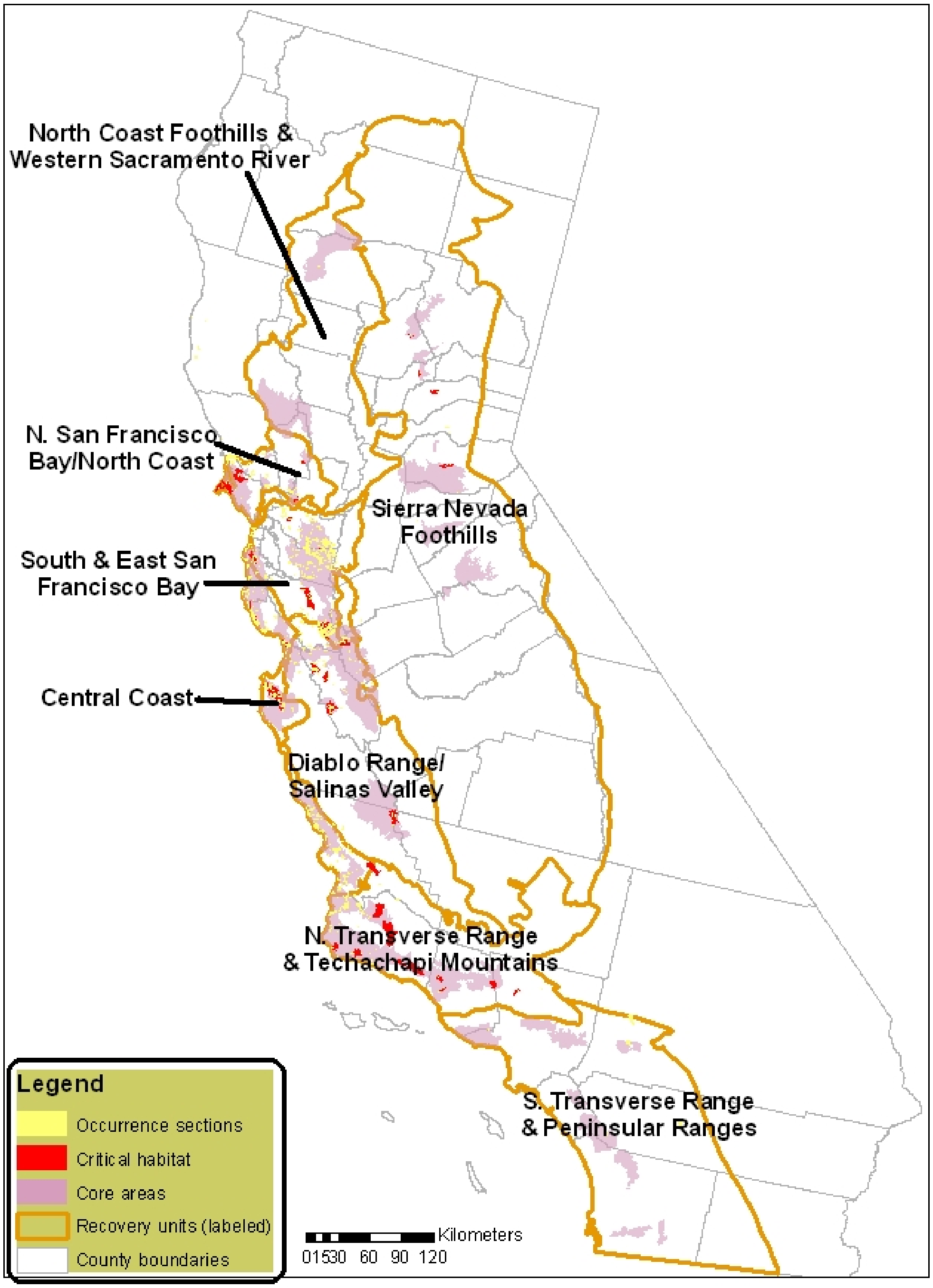
[1] Recovery units designated by the USFWS (USFWS 2000, pg 49)
[2] Core areas designated by the USFWS (USFWS 2000, pg 51)
[3] Critical habitat units designated by the USFWS on April 13, 2006 (USFWS 2006, 71 FR 19244-19346)
[4] Currently occupied (post-1985) and historically occupied core areas as designated by the USFWS (USFWS 2002, pg 54)
[5] Critical habitat unit where identified threats specifically included pesticides or agricultural runoff (USFWS
[6] Critical habitat units that are outside of core areas, but within recovery units
[7] Currently occupied core areas that are included in this effects determination are bolded.

Other Known Occurrences from the CNDBB

The CNDDB provides location and natural history information on species found in California. The CNDDB serves as a repository for historical and current species location sightings. Information regarding known occurrences of CRLFs outside of the currently occupied core areas and designated critical habitat is considered in defining the current range of the CRLF. See: http://www.dfg.ca.gov/bdb/html/cnddb_info.html for additional information on the CNDDB.

The distribution of all known occurrences of the CRLF with critical habitat, core areas, and FWS recovery unit boundaries are presented in **Figure 2.a**.

CRLF Habitat Areas



Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

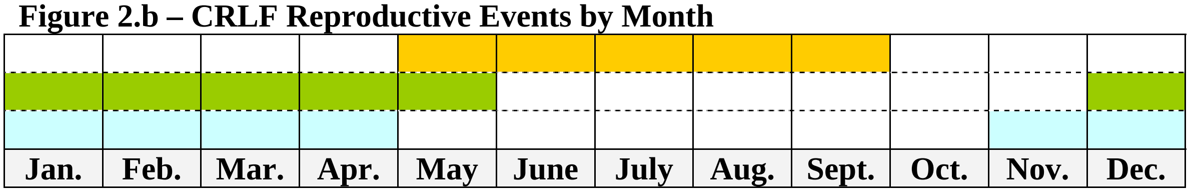
Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June 15, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure 2.a. Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF

2.5.2 Reproduction

CRLFs breed primarily in ponds; however, they may also breed in quiescent streams, marshes, and lagoons (Fellers 2005a). According to the Recovery Plan (USFWS 2002), CRLFs breed from November through late April. Peaks in spawning activity vary geographically; Fellers (2005b) reports peak spawning as early as January in parts of coastal central California. Eggs are fertilized as they are being laid. Egg masses are typically attached to emergent vegetation, such as bulrushes (*Scirpus* spp.) and cattails (*Typha* spp.) or roots and twigs, and float on or near the surface of the water (Hayes and Miyamoto 1984). Egg masses contain approximately 2000 to 6000 eggs ranging in size between 2 and 2.8 mm (Jennings and Hayes 1994). Embryos hatch 10 to 14 days after fertilization (Fellers 2005a) depending on water temperature. Egg predation is reported to be infrequent and most mortality is associated with the larval stage (particularly through predation by fish); however, predation on eggs by newts has also been reported (Rathburn 1998). Tadpoles require 11 to 28 weeks to metamorphose into juveniles (terrestrial-phase), typically between May and September (Jennings and Hayes 1994, USFWS 2002); tadpoles have been observed to over-winter (delay metamorphosis until the following year) (Fellers 2005b, USFWS 2002). Males reach sexual maturity at 2 years, and females reach sexual maturity at 3 years of age; adults have been reported to live 8 to 10 years (USFWS 2002). Figure 2.b depicts CRLF annual reproductive timing.

Figure 2.b – CRLF Reproductive Events by Month



Light Blue = **Breeding/Egg Masses**
Green = **Tadpoles (except those that over-winter)**
Orange = **Young Juveniles**
Adults and juveniles can be present all year

2.5.3 Diet

Although the diet of CRLF aquatic-phase larvae (tadpoles) has not been studied specifically, it is assumed that their diet is similar to that of other frog species, with the aquatic phase feeding exclusively in water and consuming diatoms, algae, and detritus (USFWS 2002). Tadpoles filter and entrap suspended algae (Seale and Beckvar, 1980) via mouthparts designed for effective grazing of periphyton (Wassersug, 1984, Kupferberg *et al.*; 1994; Kupferberg, 1997; Altig and McDiarmid, 1999).

Juvenile and adult CRLFs forage in aquatic and terrestrial habitats, and their diet differs greatly from that of larvae. The main food source for juvenile aquatic- and terrestrial-phase CRLFs is thought to be aquatic and terrestrial invertebrates found along the shoreline and on the water surface. Hayes and Tennant (1985) report, based on a study examining the gut content of 35 juvenile and adult CRLFs, that the species feeds on as many as 42 different invertebrate taxa, including Arachnida, Amphipoda, Isopoda, Insecta, and Mollusca. The most commonly observed prey species were larval alderflies (*Sialis* cf. *californica*), pillbugs (*Armadilliadrium vulgare*), and water striders (*Gerris* sp). The preferred prey species, however, was the sowbug (Hayes and

Tennant, 1985). This study suggests that CRLFs forage primarily above water, although the authors note other data reporting that adults also feed under water, are cannibalistic, and consume fish. For larger CRLFs, over 50% of the prey mass may consists of vertebrates such as mice, frogs, and fish, although aquatic and terrestrial invertebrates were the most numerous food items (Hayes and Tennant 1985). For adults, feeding activity takes place primarily at night; for juveniles feeding occurs during the day and at night (Hayes and Tennant 1985).

2.5.4 Habitat

CRLFs require aquatic habitat for breeding, but also use other habitat types including riparian and upland areas throughout their life cycle. CRLF use of their environment varies; they may complete their entire life cycle in a particular habitat or they may utilize multiple habitat types. Overall, populations are most likely to exist where multiple breeding areas are embedded within varying habitats used for dispersal (USFWS 2002). Breeding sites include streams, deep pools, backwaters within streams and creeks, ponds, marshes, sag ponds (land depressions between fault zones that have filled with water), dune ponds, and lagoons. Breeding adults have been found near deep (0.7 m) still or slow moving water surrounded by dense vegetation (USFWS 2002); however, the largest number of tadpoles have been found in shallower pools (0.26 – 0.5 m) (Reis, 1999).

CRLFs also frequently breed in artificial impoundments such as stock ponds, although additional research is needed to identify habitat requirements within artificial ponds (USFWS 2002). Adult CRLFs use dense, shrubby, or emergent vegetation closely associated with deep-water pools bordered with cattails and dense stands of overhanging vegetation (http://www.fws.gov/endangered/features/rl_frog/rlfrog.html#where).

In general, dispersal and habitat use depends on climatic conditions, habitat suitability, and life stage. Adults rely on riparian vegetation for resting, feeding, and dispersal. The foraging quality of the riparian habitat depends on moisture, composition of the plant community, and presence of pools and backwater aquatic areas for breeding. CRLFs can be found living within streams at distances up to 3 km (2 miles) from their breeding site and have been found up to 30 m (100 feet) from water in dense riparian vegetation for up to 77 days (USFWS 2002).

During dry periods, the CRLF is rarely found far from water, although it will sometimes disperse from its breeding habitat to forage and seek other suitable habitat under downed trees or logs, industrial debris, and agricultural features (UWFWS 2002). According to Jennings and Hayes (1994), CRLFs also use small mammal burrows and moist leaf litter as habitat. In addition, CRLFs may also use large cracks in the bottom of dried ponds as refugia; these cracks may provide moisture for individuals avoiding predation and solar exposure (Alvarez 2000).

2.6 Designated Critical Habitat

In a final rule published on April 13, 2006, 34 separate units of critical habitat were designated for the CRLF by USFWS (USFWS 2006; FR 51 19244-19346). A summary of the 34 critical habitat units relative to USFWS-designated recovery units and core areas (previously discussed in Section 2.5.1) is provided in **Table 2.4**.

‘Critical habitat’ is defined in the ESA as the geographic area occupied by the species at the time of the listing where the physical and biological features necessary for the conservation of the species exist, and there is a need for special management to protect the listed species. It may also include areas outside the occupied area at the time of listing if such areas are ‘essential to the conservation of the species.’ All designated critical habitat for the CRLF was occupied at the time of listing. Critical habitat receives protection under Section 7 of the ESA through prohibition against destruction or adverse modification with regard to actions carried out, funded, or authorized by a federal Agency. Section 7 requires consultation on federal actions that are likely to result in the destruction or adverse modification of critical habitat.

To be included in a critical habitat designation, the habitat must be ‘essential to the conservation of the species.’ Critical habitat designations identify, to the extent known using the best scientific and commercial data available, habitat areas that provide essential life cycle needs of the species or areas that contain certain primary constituent elements (PCEs) (as defined in 50 CFR 414.12(b)). PCEs include, but are not limited to, space for individual and population growth and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, rearing (or development) of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species. The designated critical habitat areas for the CRLF are considered to have the following PCEs that justify critical habitat designation:

- Breeding aquatic habitat;
- Non-breeding aquatic habitat;
- Upland habitat; and
- Dispersal habitat.

Please note that a more complete description of these habitat types is provided in **Attachment 1**.

Occupied habitat may be included in the critical habitat only if essential features within the habitat may require special management or protection. Therefore, USFWS does not include areas where existing management is sufficient to conserve the species. Critical habitat is designated outside the geographic area presently occupied by the species only when a designation limited to its present range would be inadequate to ensure the conservation of the species. For the CRLF, all designated critical habitat units contain all four of the PCEs, and were occupied by the CRLF at the time of FR listing notice in April 2006. The FR notice designating critical habitat for the CRLF includes a special rule exempting routine ranching activities associated with livestock ranching from incidental take prohibitions. The purpose of this exemption is to promote the conservation of rangelands, which could be beneficial to the CRLF, and to reduce the rate of conversion to other land uses that are incompatible with CRLF conservation. Please see **Attachment 1** for a full explanation on this special rule.

USFWS has established adverse modification standards for designated critical habitat (USFWS 2006). Activities that may destroy or adversely modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to use of azinphos methyl that may alter the PCEs of the CRLF’s critical habitat form the basis of the critical habitat impact analysis. According to USFWS (2006), activities that may affect critical habitat and therefore result in adverse effects to the CRLF include, but are not limited to the following:

(1) Significant alteration of water chemistry or temperature to levels beyond the tolerances of the CRLF that result in direct or cumulative adverse effects to individuals and their life-cycles.
(2) Significant increase in sediment deposition within the stream channel or pond or disturbance of upland foraging and dispersal habitat that could result in elimination or reduction of habitat necessary for the growth and reproduction of the CRLF by increasing the sediment deposition to levels that would adversely affect their ability to complete their life cycles.
(3) Significant alteration of channel/pond morphology or geometry that may lead to changes to the hydrologic functioning of the stream or pond and alter the timing, duration, water flows, and levels that would degrade or eliminate the CRLF and/or its habitat. Such an effect could also lead to increased sedimentation and degradation in water quality to levels that are beyond the CRLF's tolerances.
(4) Elimination of upland foraging and/or aestivating habitat or dispersal habitat.
(5) Introduction, spread, or augmentation of non-native aquatic species in stream segments or ponds used by the CRLF.
(6) Alteration or elimination of the CRLF's food sources or prey base (also evaluated as indirect effects to the CRLF).

As previously noted in Section 2.1, the Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitat. Because azinphos methyl is expected to directly impact living organisms within the action area, critical habitat analysis for azinphos methyl is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked to biologically mediated processes.

2.7 Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the federal action and not merely the immediate area involved in the action (50 CFR 402.02). It is recognized that the overall action area for the national registration of azinphos methyl is likely to encompass considerable portions of the United States based on the large array of agricultural uses. However, the scope of this assessment limits consideration of the overall action area to those portions that may be applicable to the protection of the CRLF and its designated critical habitat within the state of California. Deriving the geographical extent of this portion of the action area is the product of consideration of the types of effects that azinphos methyl may be expected to have on the environment, the exposure levels to azinphos methyl that are associated with those effects, and the best available information concerning the use of azinphos methyl and its fate and transport within the state of California.

The definition of action area requires a stepwise approach that begins with an understanding of the federal action. The federal action is defined by the currently labeled uses for azinphos methyl. An analysis of labeled uses and review of available product labels was completed. This analysis indicates that, for azinphos methyl, the following uses are considered as part of the federal action evaluated in this assessment:

- Almonds
- Apples
- Blueberries (Low- and Highbush)
- Brussels Sprouts
- Cherries (Sweet and Tart)
- Nursery Stock
- Parsley
- Pears
- Pistachios
- Walnuts

According to the label, two of these azinphos methyl uses, parsley and blueberries, may not be used in California. Thus, parsley and blueberries will not be considered in this assessment because they do not result in exposure to the CRLF.

After determination of which uses will be assessed, an evaluation of the potential “footprint” of the use pattern should be determined. This “footprint” represents the initial area of concern and is typically based on available land cover data. Local land cover data available for the state of California were analyzed to refine the understanding of potential azinphos methyl use. The overall conclusion of this analysis is that the action area is limited to agricultural lands only and given that all non-agricultural uses have been cancelled these use sites need not be considered in defining the action area. The initial area of concern is defined as all land cover types that represent the labeled uses described above. A map representing all the land cover types that make up the initial area of concern is presented in **Figure 2.c**.

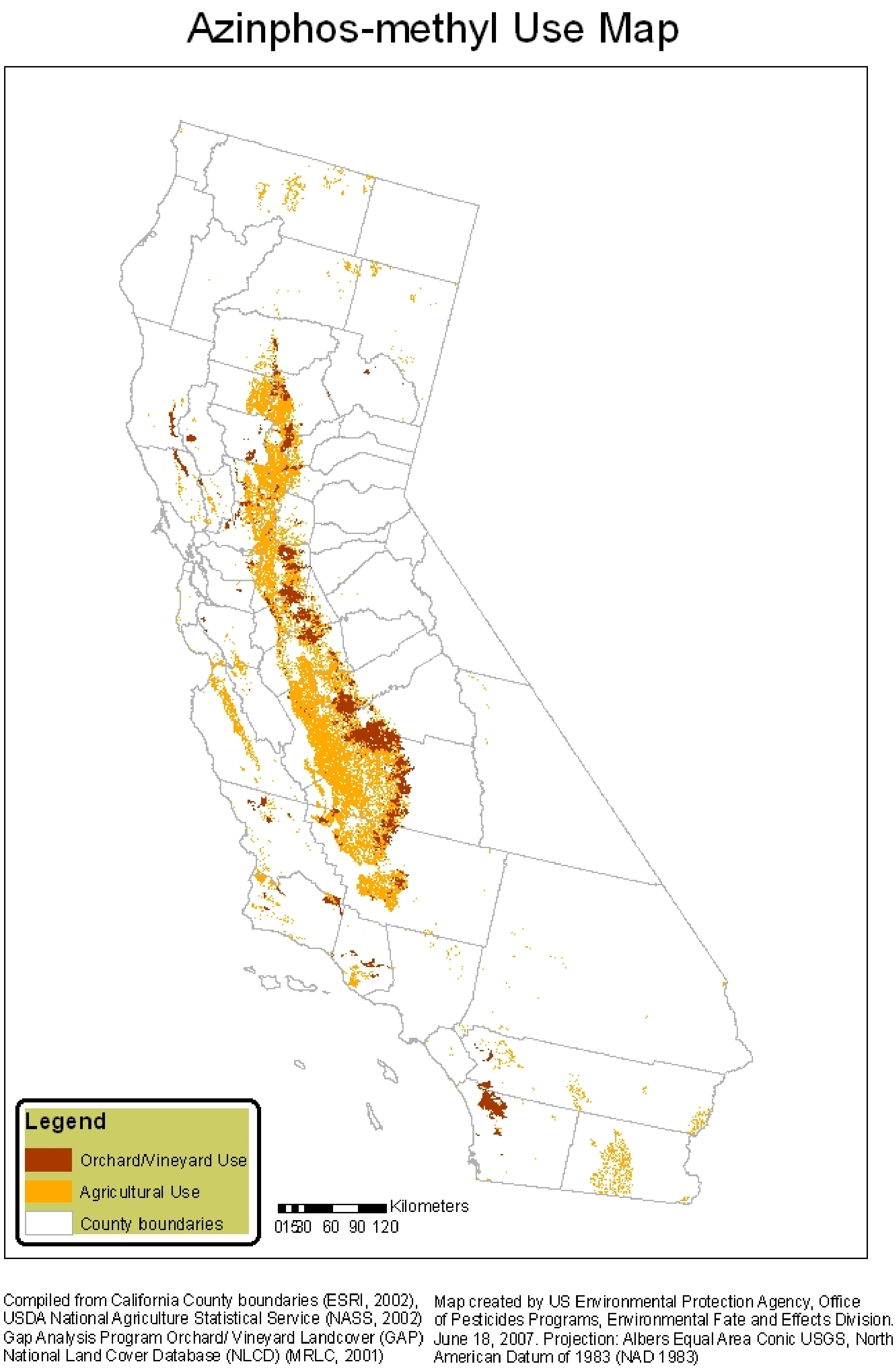


Figure 2.c. Map of land cover use sites making up the intital area of concern, or "footprint" of potential use, for azinphos methyl.

Once the initial area of concern is defined, the next step is to compare the extent of that area with the results of the screening level risk assessment. The screening level risk assessment will define which taxa, if any, are predicted to be exposed at concentrations above the Agency's Levels of Concern (LOC). The screening level assessment includes an evaluation of the environmental fate properties of azinphos methyl to determine which routes of transport are likely to have an impact on the CRLF.

For azinphos methyl the principal routes of transport away from the application site are expected to be runoff and spray drift due to its mobility and moderate persistence. However, azinphos methyl has also been documented to occur in air monitoring samples, and thus, long-range transport away from the area of application cannot be precluded. Typically, air monitoring studies do not distinguish the route of transport associated with the detections. The location of the available air monitoring for azinphos methyl in Glenn and Kern counties suggest that these detections are related to nearby sources and are more likely due to spray drift than long-range transport. In addition, sampling from the high Sierra Nevada Mountains has not detected azinphos methyl. Furthermore, the vapor pressure of azinphos methyl suggests that volatilization leading to long-range transport is unlikely, as mentioned previously (Section 2.4.2).

LOC exceedances are used to describe how far effects may be seen from the initial area of concern. Factors considered include: spray drift, downstream run-off, atmospheric transport, etc. Typically, this information is incorporated into GIS and a map of the action area is created.

AgDRIFT modeling can be used to define how far from the initial area of concern an effect to a given species may be expected. A spray drift analysis for azinphos methyl using the most sensitive toxicity endpoints (*i.e.*, aquatic and terrestrial invertebrate acute mortality data) suggests that the distance for potential effects from the treated area of concern is beyond the range of the AgDrift model (*i.e.*, 2608 feet). Subsequently, the AgDISP model with the Gaussian extension (for longer range transport) was used to define this distance. The AgDISP model was run in aerial mode in order to mimic airblast applications because the AgDISP model does not have an airblast mode. AgDisp was run with the following settings beyond the standard default settings:

- 30 gal/acre spray volume rate (limit of the model)
- a 15 ft release height (label specific)
- 10 mph wind speed (label specific)
- very fine to fine spray spectrum (label specifies larger droplet sizes for groundboom applications, but not for airblast spray), and
- no canopy.

For azinphos methyl, analyses of the spray drift buffers needed to get below concentrations that exceed the LOC have been conducted for both aquatic and terrestrial endpoints. Specifically, the most sensitive endpoint for aquatic species (freshwater invertebrate) and terrestrial species (terrestrial invertebrates) were used to define a concentration below which the LOC would not be exceeded. For both freshwater and terrestrial invertebrates, the most sensitive toxicity value was multiplied by the LOC (0.05 for endangered species) to yield an exposure concentration that would result in an RQ below the LOC. The analysis (**Appendix A**) indicated that aquatic buffers need to be 685 feet while the terrestrial buffers need to be 3707 feet to reduce concentrations from spray drift alone to below the LOC.

Using the ratio of LOC to the highest RQ for terrestrial species (0.05/817 = 6.12×10^{-5}) multiplied by the single application rate for the use on apples (1.5 lbs a.i./A or 1.68 kg a.i./ha) yields an initial average deposition of 1.03×10^{-4} kg/ha. Using this value as input into the AgDISP model exceeds the limits of calculation, but incorporation of the Gaussian extension yields a buffer of 3707 feet. For the aquatic buffer distance, the LOC/RQ is 0.00127 (0.05/39--for freshwater invertebrates for the almond use). Using this value as input into the AgDISP model yields at buffer of 685 feet (no Gaussian extension needed). Given that the greatest buffer distance was 3707 feet (for terrestrial invertebrates), this value was used to buffer the initial area of concern (Figure 2.d) as the first step in defining the final action area.

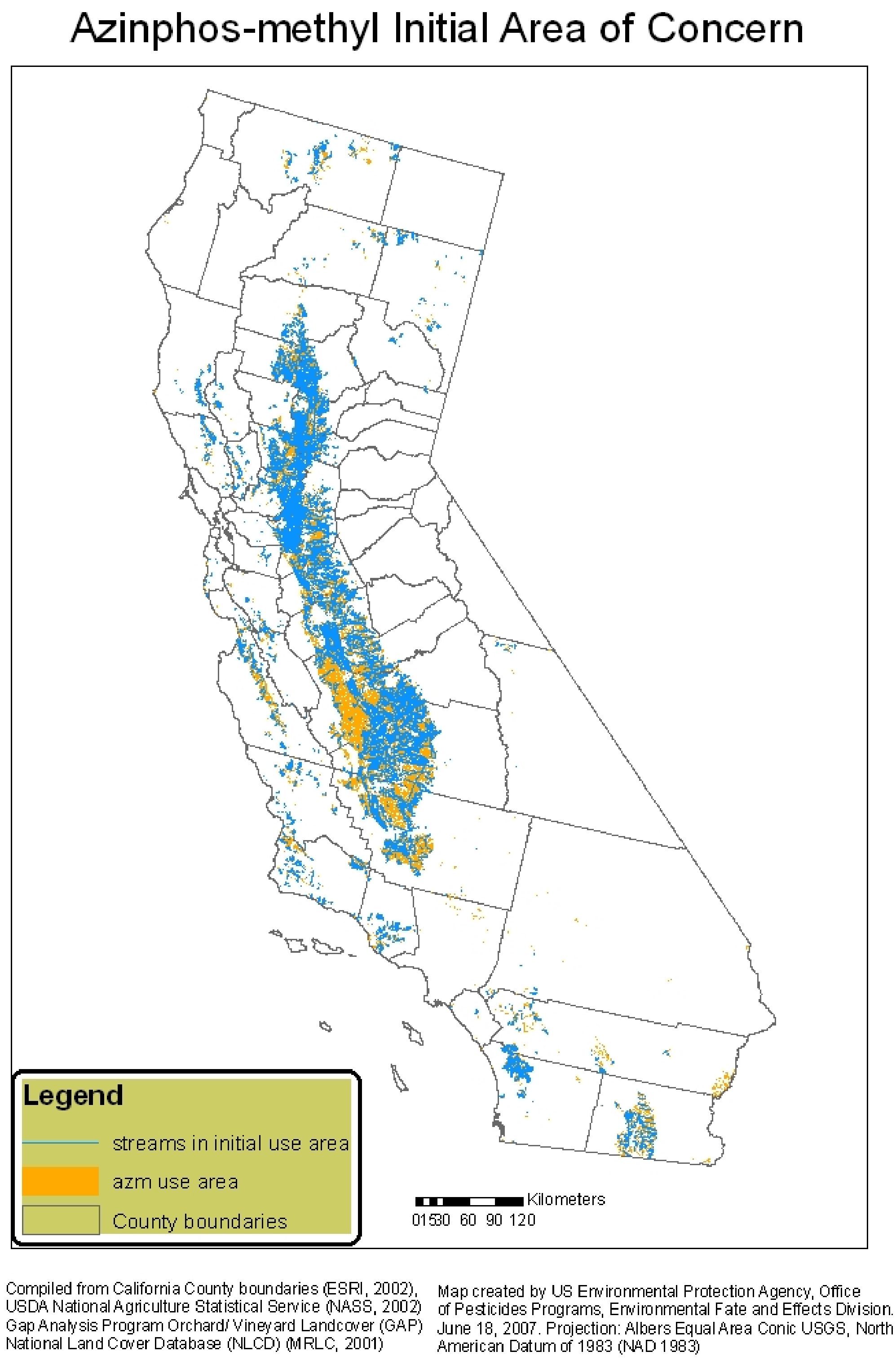


Figure 2.d. Initial area of concern or "footprint" of potential azinphos methyl use.

The final step in defining the action area was to determine the downstream extent of exposure in streams and rivers where the EEC could potentially be above levels that would exceed the most sensitive LOC. To complete this assessment, the greatest ratio of aquatic RQ to LOC was estimated. As above, this ratio was 788 (39/0.05). Using the downstream dilution approach (described in more detail in **Appendix A**) yields a target percent crop area (PCA) of 0.13%. This value has been input into the downstream dilution approach and a total of 194 kilometers of stream downstream from the initial area of concern (footprint of use). By way of comparison there are 30,419 kilometers of streams within the intitial area of concern all of which are assumed to be at the modeled EEC.

The initial area of concern plus the buffered area plus the downstream extent yields the final action area for azinphos methyl use in California. The action area is presented graphically for the whole state of California in **Figure 2e.**

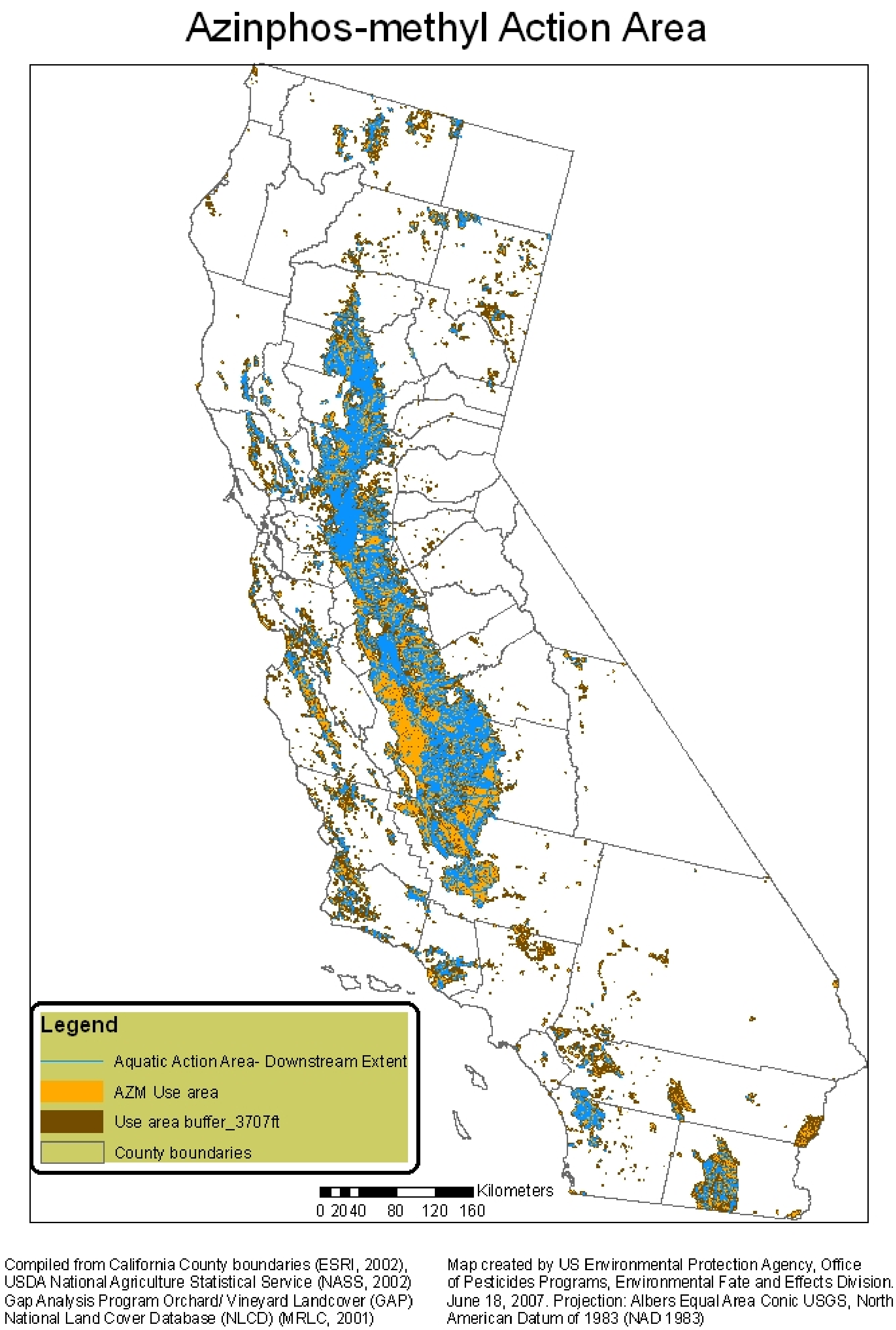


Figure 2.e. Azinphos methyl action area for the California Red Legged Frog assessment.

Two additional steps are conducted subsequent to defining the action area and used to characterize the potential for effects. The first is an evaluation of the overlap between the action area as defined above and the species range defined in Section 2.5.1. The complete description of this analysis is provided in Appendix A, including regionalized maps showing where co-occurrence of action area and species are found and a summary of areas of overlap. These data are provided both at the state level and within Recovery Units. The characterization by Recovery Unit is intended to provide context to where the effects determinations are spatially relevant, but does not necessarily impact the effects determination itself.

The second piece of characterization involved an evaluation of usage information to determine areas where use of azinphos methyl may impact the CRLF. This analysis involves identifying where azinphos methyl has been reportedly used and on what crops. This analysis is used to characterize where predicted exposures are most likely to occur but does not preclude use in other portions of the action area. A more detailed review of the county-level use information was also completed. These data suggest that azinphos methyl has historically been used on a wide variety of crop and non-crop uses, but the majority of the use has focused on orchard crops, which are part of this assessment. Additional analysis of the dominant location and typical rates associated with these orchard uses is described in the risk characterization section of this assessment.

Both the analysis of overlap between species and action area and the significance of the CDPR PUR use data are summarized in **Section 6.1.**

2.8 Assessment Endpoints and Measures of Ecological Effect

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected."[4] Selection of the assessment endpoints is based on valued entities (e.g., CRLF, organisms important in the life cycle of the CRLF, and the PCEs of its designated critical habitat), the ecosystems potentially at risk (e.g.,. waterbodies, riparian vegetation, and upland and dispersal habitats), the migration pathways of azinphos methyl (e.g., runoff, spray drift, etc.), and the routes by which ecological receptors are exposed to azinphos methyl-related contamination (e.g., direct contact, etc).

2.8.1. Assessment Endpoints for the CRLF

Assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the CRLF, as well as indirect effects, such as reduction of the prey base and/or modification of its habitat. In addition, potential destruction and/or adverse modification of critical habitat is assessed by evaluating potential effects to PCEs, which are components of the habitat areas that provide essential life cycle needs of the CRLF. Each assessment endpoint requires one or more "measures of ecological effect," defined as changes in the attributes of an assessment endpoint or changes in a surrogate entity or attribute in response to exposure to a pesticide. Specific measures of ecological effect are generally evaluated based on acute and chronic toxicity information from registrant-submitted guideline tests that are performed on a limited number of organisms. Additional ecological effects data from the open literature are also considered.

[4] U.S. EPA (1992). *Framework for Ecological Risk Assessment.* EPA/630/R-92/001.

A complete discussion of all the toxicity data available for this risk assessment, including resulting measures of ecological effect selected for each taxonomic group of concern, is included in Section 4 of this document. A summary of the assessment endpoints and measures of ecological effect selected to characterize potential assessed direct and indirect CRLF risks associated with exposure to azinphos methyl is provided in **Table 2.5**.

Table 2.5 Summary of Assessment Endpoints and Measures of Ecological Effects for Direct and Indirect Effects of azinphos methyl on the California Red-legged Frog

Assessment Endpoint	Measures of Ecological Effects
Aquatic Phase (eggs, larvae, tadpoles, juveniles, and adults)[a]	
1. Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	1a. Fowlers toad (*Bufo fowleri*) LC_{50} = 109 µg a.i./L 1b. Fowlers toad (*Bufo fowleri*) estimated NOAEC[b] = 16.5 µg a.i./L
2. Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	2a. Northern pike acute LC_{50} = 0.36 µg a.i./L 2b. *Gammarus fasciatus* acute LC_{50} = 0.16 µg a.i./L 2c. Northern pike estimated NOAEC[b] = 0.055 µg a.i./L 2d. *Gammarus fasciatus* estimated NOAEC[b] = 0.036 µg a.i./L
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	3a. Vascular plant acute EC_{50} – no data available 3b. Non-vascular plant acute EC_{50} – no data available
4. Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	4a. Distribution of EC_{25} values for monocots – no data available 4b. Distribution of EC_{25} values for dicots[5] – no data available
Terrestrial Phase (Juveniles and adults)	
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	5a. Northern bobwhite quail[c] acute oral LD_{50} = 32 mg a.i./kg 5b. Northern bobwhite quail[c] subacute dietary LC_{50} = 488 ppm 5c. Mallard duck[c] chronic reproduction NOAEC = 10.5 ppm
6. Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial vertebrates, including mammals and terrestrial phase amphibians)	6a. Honeybee acute contact LD_{50} = 0.063 µg a.i./L = 0.491 ppm[d] 6b. Lab rat acute oral LD_{50} = 7.8 mg a.i./kg 6c. Gray-tailed vole subacute dietary LC_{50} = 406 ppm 6d. Lab rat developmental and chronic NOAEC = 5 ppm
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation)	7a. Distribution of EC_{25} for monocots – no data available 7b. Distribution of EC_{25} for dicots – no data available

[a] Adult frogs are no longer in the "aquatic phase" of the amphibian life cycle; however, submerged adult frogs are considered "aquatic" for the purposes of this assessment because exposure pathways in the water are considerably different that exposure pathways on land.
[b] Estimated using the acute-to-chronic ratio
[c] Birds are used as surrogates for terrestrial phase amphibians.
[d] According to Mayer, D. & C. Johansen. 1990. *Pollinator Protection: A Bee & Pesticide Handbook*. Wicwas Press. Cheshire, Conn. p. 161

2.8.2. Assessment Endpoints for Designated Critical Habitat

As previously discussed, designated critical habitat is assessed to evaluate actions related to the use of azinphos methyl that may alter the PCEs of the CRLF's critical habitat. PCEs for the CRLF were previously described in Section 2.6. Actions that may destroy or adversely modify critical habitat are those that alter the PCEs of the CRLF. Therefore, these actions are identified as

[5] The available information indicates that the California red-legged frog does not have any obligate relationships.

assessment endpoints. It should be noted that evaluation of PCEs as assessment endpoints is limited to those of a biological nature (i.e., the biological resource requirements for the listed species associated with the critical habitat) and those for which azinphos methyl effects data are available.

Assessment endpoints and measures of ecological effect selected to characterize potential modification to designated critical habitat associated with exposure to azinphos methyl are provided in **Table 2.6**. Adverse modification to the critical habitat of the CRLF includes the following, as specified by USFWS (2006) and previously discussed in Section 2.6:

1. Alteration of water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs.
2. Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.
3. Significant increase in sediment deposition within the stream channel or pond or disturbance of upland foraging and dispersal habitat.
4. Significant alteration of channel/pond morphology or geometry.
5. Elimination of upland foraging and/or aestivating habitat, as well as dispersal habitat.
6. Introduction, spread, or augmentation of non-native aquatic species in stream segments or ponds used by the CRLF.
7. Alteration or elimination of the CRLF's food sources or prey base.

Some components of these PCEs are associated with physical abiotic features (e.g., presence and/or depth of a water body, or distance between two sites), which are not expected to be measurably altered by use of pesticides. Assessment endpoints used for the analysis of designated critical habitat are based on the adverse modification standard established by USFWS (2006).

<table>
<tr><th colspan="2">Table 2.6. Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat</th></tr>
<tr><th>Assessment Endpoint</th><th>Measures of Ecological Effect</th></tr>
<tr><td colspan="2">Aquatic Phase PCEs
(Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)</td></tr>
<tr><td>Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.</td><td>a. Most sensitive aquatic plant EC_{50} – no data available
b. Distribution of EC_{25} values for terrestrial monocots – no data available
c. Distribution of EC_{25} values for terrestrial dicots – no data available</td></tr>
<tr><td>Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.[6]</td><td>a. Most sensitive EC_{50} values for aquatic plants – no data available
b. Distribution of EC_{25} values for terrestrial monocots – no data available
c. Distribution of EC_{25} values for terrestrial dicots – no data available</td></tr>
<tr><td>Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.</td><td>a. Northern pike acute LC_{50} = 0.36 µg a.i./L
b. Gammarus fasciatus acute LC_{50} = 0.16 µg a.i./L
c. Northern pike estimated NOAEC[a] = 0.055 µg a.i./L
d. Gammarus fasciatus estimated NOAEC[a] = 0.036 µg a.i./L</td></tr>
<tr><td>Reduction and/or modification of aquatic-based food sources for pre-metamorphs (e.g., algae)</td><td>a. Most sensitive aquatic plant EC_{50} – no data available</td></tr>
<tr><td colspan="2">Terrestrial Phase PCEs
(Upland Habitat and Dispersal Habitat)</td></tr>
<tr><td>Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance</td><td rowspan="4">a. Distribution of EC_{25} values for monocots – no data available
b. Distribution of EC_{25} values for dicots– no data available
c. Lab rat acute oral LD_{50} = 7.8 mg a.i./kg, Gray-tailed vole subacute dietary LC_{50} = 406 ppm, and developmental and chronic NOAEC = 5 ppm
d. Northern pike acute LC_{50} = 0.36 µg a.i./L and Rainbow trout chronic reproduction NOAEC = 0.44 µg a.i./L
e. Northern bobwhite quail acute oral LD_{50} = 32 mg a.i./kg, Northern bobwhite quail subacute dietary LC_{50} = 488 ppm, and mallard duck chronic reproduction NOAEC = 10.5 ppm
f. Honeybee acute contact LD_{50} = 0.063 µg a.i./L = 0.491 ppm[b]</td></tr>
<tr><td>Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal</td></tr>
<tr><td>Reduction and/or modification of food sources for terrestrial phase juveniles and adults</td></tr>
<tr><td>Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.</td></tr>
<tr><td colspan="2">[a] Estimated using the acute-to-chronic ratio
[b] According to Mayer, D. & C. Johansen. 1990. Pollinator Protection: A Bee & Pesticide Handbook. Wicwas Press. Cheshire, Conn. p. 161</td></tr>
</table>

[6] Physico-chemical water quality parameters such as salinity, pH, and hardness are not evaluated because these processes are not biologically mediated and, therefore, are not relevant to the endpoints included in this assessment.

2.9 Conceptual Model

2.9.1 Risk Hypotheses

Risk hypotheses are specific assumptions about potential adverse effects (i.e., changes in assessment endpoints) and may be based on theory and logic, empirical data, mathematical models, or probability models (U.S. EPA, 1998). For this assessment, the risk is stressor-linked, where the stressor is the release of azinphos methyl to the environment. The following risk hypotheses are presumed for this endangered species assessment:

- Labeled uses of azinphos methyl within the action area may directly affect the CRLF by causing mortality or by adversely affecting growth or fecundity;
- Labeled uses of azinphos methyl within the action area may indirectly affect the CRLF by reducing or changing the composition of food supply;
- Labeled uses of azinphos methyl within the action area may indirectly affect the CRLF and/or adversely modify designated critical habitat by reducing or changing the composition of the aquatic plant community in the ponds and streams comprising the species' current range and designated critical habitat, thus affecting primary productivity and/or cover;
- Labeled uses of azinphos methyl within the action area may indirectly affect the CRLF and/or adversely modify designated critical habitat by reducing or changing the composition of the terrestrial plant community (i.e., riparian habitat) required to maintain acceptable water quality and habitat in the ponds and streams comprising the species' current range and designated critical habitat;
- Labeled uses of azinphos methyl within the action area may adversely modify the designated critical habitat of the CRLF by reducing or changing breeding and non-breeding aquatic habitat (via modification of water quality parameters, habitat morphology, and/or sedimentation);
- Labeled uses of azinphos methyl within the action area may adversely modify the designated critical habitat of the CRLF by reducing the food supply required for normal growth and viability of juvenile and adult CRLFs;
- Labeled uses of azinphos methyl within the action area may adversely modify the designated critical habitat of the CRLF by reducing or changing upland habitat within 200 ft of the edge of the riparian vegetation necessary for shelter, foraging, and predator avoidance.
- Labeled uses of azinphos methyl within the action area may adversely modify the designated critical habitat of the CRLF by reducing or changing dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal.
- Labeled uses of azinphos methyl within the action area may adversely modify the designated critical habitat of the CRLF by altering chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.

2.9.2 Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the stressor (azinphos methyl), release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for aquatic and terrestrial phases of the CRLF are shown in Figures 2.f and 2.g, and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in Figures 2.h and 2.i. Exposure routes shown in dashed lines are not quantitatively considered because the resulting exposures are expected to be so low as not to cause adverse effects to the CRLF.

The general conceptual model of exposure for the CRLF is expected to be dominated by runoff and spray drift. Azinphos methyl is not expected to leach to groundwater and thus this route of exposure is not considered significant. In addition, long-range transport beyond spray drift was evaluated and based on the vapor pressure and available monitoring data is not considered a significant route of exposure.

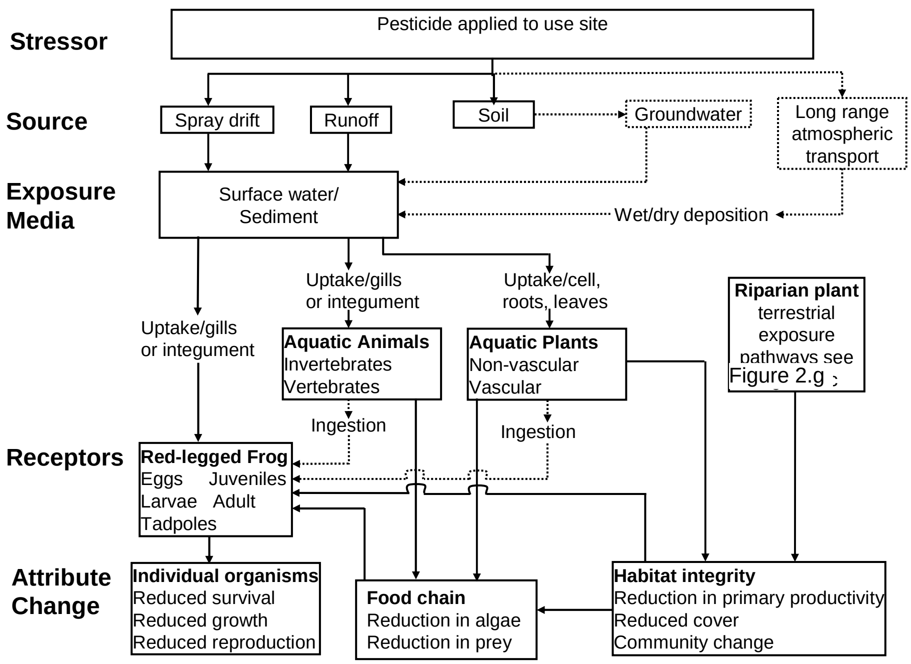


Figure 2.f Conceptual Model for Azinphos Methyl Effects on Aquatic Phase of the Red-Legged Frog

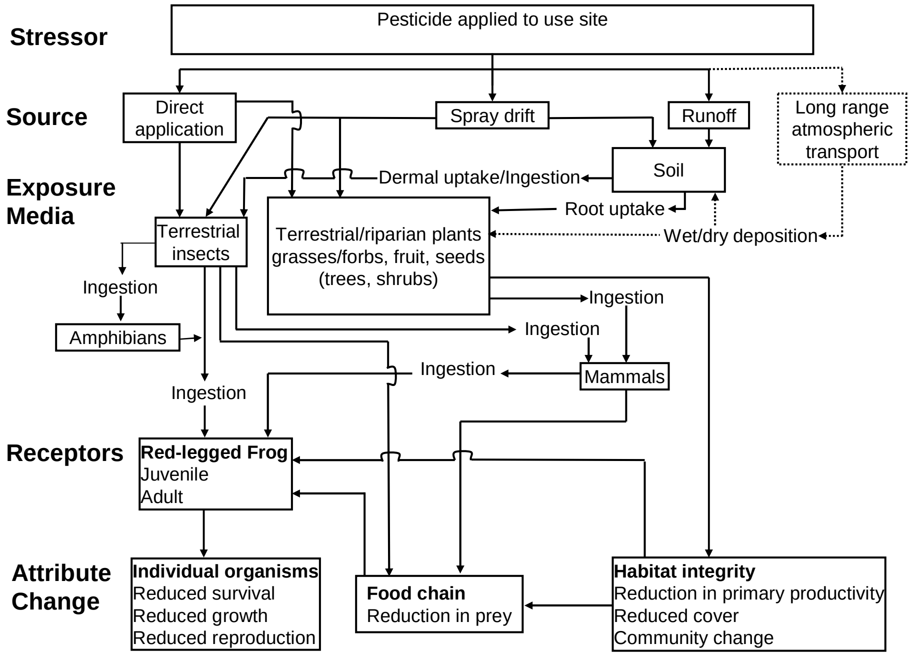


Figure 2.g Conceptual Model for Azinphos Methyl Effects on Terrestrial Phase of Red-Legged Frog

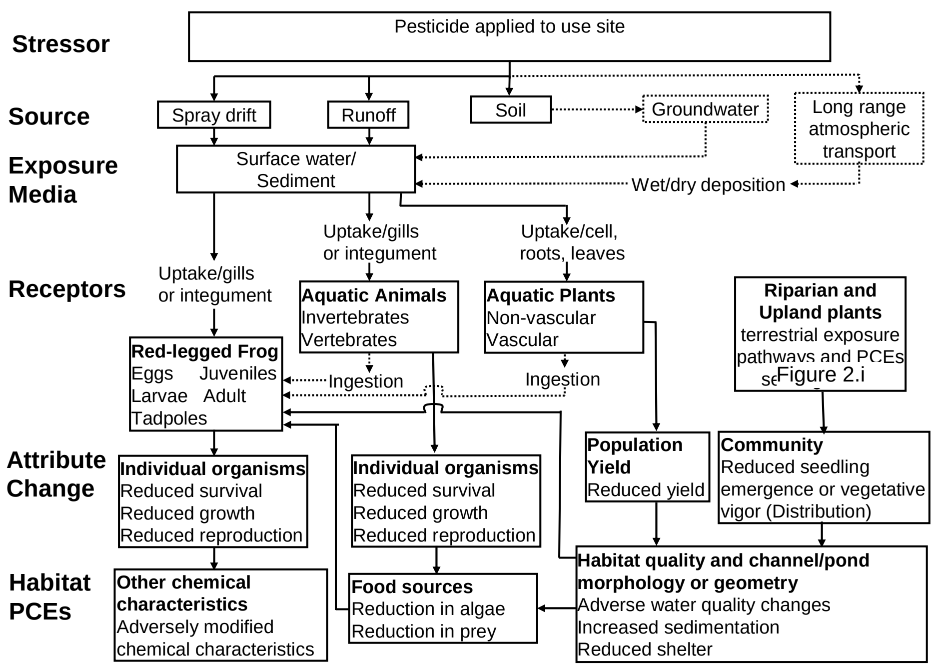


Figure 2.h Conceptual Model for Azinphos Methyl Effects on Aquatic Components of Red-Legged Frog Critical Habitat

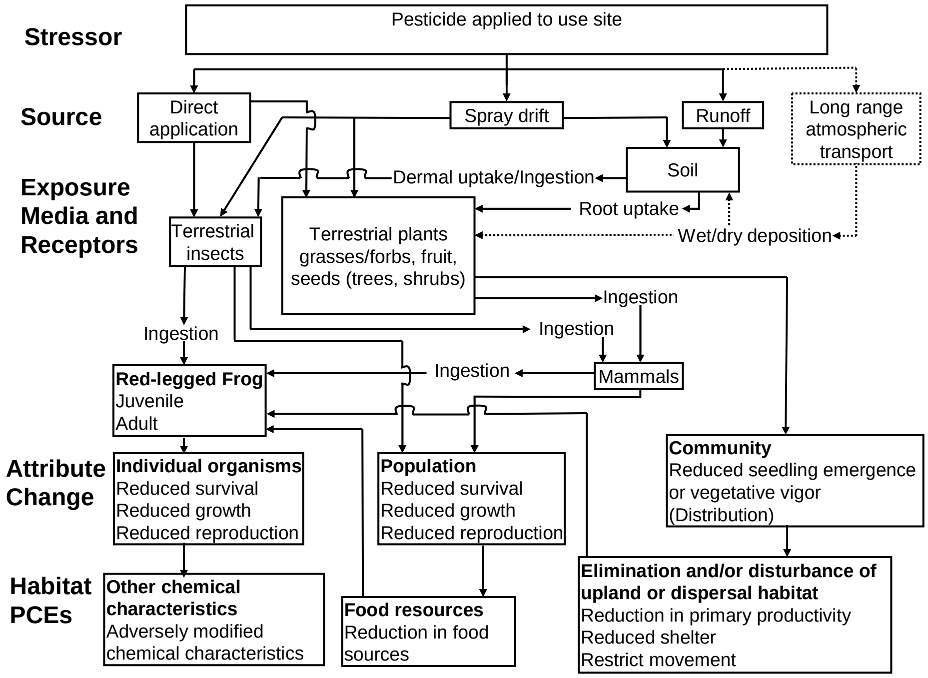


Figure 2.i Conceptual Model for Azinphos Methyl Effects on Terrestrial Components of Red-Legged Frog Critical Habitat

2.10 Analysis Plan

Potential effects of azinphos methyl to the CRLF will be estimated quantitatively using a deterministic risk quotient approach based on application information provided on the product labels. Potential exposure pathways (*i.e.*, runoff, spray drift, dietary residues on vegetation and insects) result from ground applications of aqueous solutions of azinphos methyl to agricultural crops.

Environmental exposures can be estimated from monitoring data or by simulation modeling. In this assessment, measures of exposure for azinphos methyl will be made primarily with simulation modeling, which are supported qualitatively with monitoring data. The aquatic exposure assessment for azinphos methyl is based on the Tier II simulation models PRZM and EXAMS (http://epa.gov/oppefed1/models/water/index.htm). Terrestrial wildlife exposure estimates are typically calculated for bird and mammals, which are surrogates for terrestrial-phase amphibians and reptiles. These estimates focus on potential dietary exposures to the pesticide active ingredient and are estimated assuming that organisms are exposed to a single pesticide residue on food items in a given exposure scenario. Dietary residues will be modeled for mammals and birds (*e.g.*, vegetation, insects, seeds) using the conceptual approach given in the model T-REX (version 1.3.1, 2006). In addition, terrestrial exposure and risk for the terrestrial-phase of the CRLF will be estimated using the T-HERPS model (version 1.0, 2007), which is a modified version of T-REX (version 1.3.1) that allows for estimation of food intake for herptiles. Birds are typically used as surrogates for reptiles and terrestrial-phase amphibians. However, reptiles and amphibians are poikilotherms (body temperature varies with environmental temperature) while birds are homeotherms (temperature is regulated, constant, and largely independent of environmental temperatures). Therefore, reptiles and amphibians (*i.e.*, herptiles) tend to have much lower metabolic rates and lower caloric intake requirements than birds or mammals. As a consequence, birds are likely to consume more food than amphibians or reptiles on a daily dietary intake basis, assuming similar caloric content of the food items. T-REX (version 1.3.1.) has been altered to allow for an estimation of food intake for herptiles (T-HERPS) using the same basic procedure that T-REX uses to estimate avian food intake (see **Appendix F** for details).

Measures of effect are based on changes in the attribute of an entity in response to a stressor and are generally based on the results of a toxicity study, although monitoring data may also be used to provide supporting lines of evidence for the risk characterization. Measures of acute effects (e.g., LC_{50}) and chronic effects (e.g., NOAEC) for aquatic and terrestrial organisms will be considered in this risk assessment. **Tables 2.5 and 2.6** summarize the toxicity endpoints that will be used to assess the effects of azinphos methyl on the CRLF.

Results of the exposure and toxicity effects data are used to evaluate the likelihood of adverse ecological effects on the CRLF. The risk quotient (RQ) method will be used to compare estimated environmental concentrations (EECs) and to measured acute and chronic toxicity values. RQs are typically calculated using the most sensitive species in a given taxonomic group; in this case, RQs calculated with other species are also discussed in the Risk Description (Section 5.2). For this CRLF risk assessment, RQs are compared to the Agency's Federally-listed endangered species levels of concern (LOCs).

As part of the risk characterization, an interpretation of acute RQ for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (*i.e.*, mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to azinphos methyl on par with the acute toxicity endpoint selected for RQ calculation. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose response relationship (see Section 4.3).

3. Exposure Assessment

3.1 Label Application Rates and Intervals

Azinphos methyl is currently registered only for ten crops, making it geographically restricted to several high use locations, including the Shenandoah and Cumberland Valleys, central Washington, Central Valley of California, and Michigan. These uses are almonds, apples, blueberries (low- and highbush), Brussels sprouts, cherries (sweet and tart), nursery stock, parsley, pears, pistachios, and walnuts. Azinphos methyl is not allowed to be used on blueberries and parsley in California; thus, these uses will not be considered in this assessment. The current label for azinphos methyl states that the product should not be applied within 25 feet of permanent water bodies, including rivers, natural ponds, lakes, streams, reservoirs, marshes, estuaries, or commercial fish ponds. Application rates and management practices for each of the assessed uses are summarized in **Table 3.1**.

Table 3.1. Azinphos methyl application rates and management practices for 2007.					
Crop	**Max. Rate (lbs a.i./A)**	**Max. No. Apps.**	**Minimum Interval (days)**	**Buffer Width (ft)**	**Method**
Almonds[1]	2	1	NA	25	air blast
Apples[1]	1.5	32	7 d	25	air blast
Brussels sprouts	0.75	1	NA	25	ground spray
Cherries[1,3]	0.75	2	14 d	25	air blast
Nursery Stock4	1	4	10 d	25	air blast
Pears[1]	1.5	2	7 d	25	air blast
Pistachios[1]	2	1	NA	25	air blast
Walnuts[1]	2	1	NA	25	air blast

[1] No dormant application allowed
[2] Last application of 1.0 lb $acre^{-1}$ as yearly maximum is 4 lb $acre^{-1}$.
[3] Several azinphos methyl products are restricted from application to cherries before harvest in California
[4]The ornamental use specifically excludes Christmas trees.

3.2 Aquatic Exposure Assessment

For tier 2 surface-water assessments, two models are used in tandem. PRZM simulates fate and transport on the agricultural field. The version of PRZM (Carsel et al., 1998) used was PRZM 3.12 beta, dated May 24, 2001. The water body is simulated with EXAMS version 2.98, dated July 18, 2002 (Burns, 1997). Tier 2 simulations are run for multiple (usually 30) years and the reported EECs are the concentrations that are expected once every ten years based on the thirty years of daily values generated by the simulation. PRZM and EXAMS were run using the PE4 shell, dated May 14, 2003, which also summarizes the output. Spray drift was simulated using the AgDrift model version 2.01 dated May 24, 2001.

3.2.1 Modeling Approach

Aquatic exposures were quantitatively estimated for all of assessed uses using scenarios that represent high exposure sites for azinphos methyl use. Each of these sites represents a 10 hectare field that drains into a 1-hectare pond that is 2 meters deep and has no outlet. Exposure estimates generated using the standard pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and first-order streams. As a group, there are factors that make these water bodies more or less vulnerable than the standard surrogate pond. Static water bodies that have larger ratios of drainage area to water body volume would be expected to have higher peak EECs than the standard pond. These water bodies will be either shallower or have large drainage areas (or both). Shallow water bodies tend to have limited additional storage capacity, and thus, tend to overflow and carry pesticide in the discharge whereas the standard pond has no discharge. As watershed size increases beyond 10 hectares, at some point, it becomes unlikely that the entire watershed is planted to a single crop, which is all treated with the pesticide. Headwater streams can also have peak concentrations higher than the standard pond, but they tend to persist for only short periods of time and are then carried downstream.

Management Practices

A buffer strip of 25 feet for permanent water bodies was evaluated, which is consistent with label requirements for almonds, apples, Brussels sprouts, cherries, pears, pistachios and walnuts. The modeled spray drift from AgDrift for the 25 ft buffer was 0.93% which is multiplied by three due to the lack of robustness of the orchard airblast data in AgDrift (USEPA, 2004b) to give a spray drift input of 2.8% for PRZM/EXAMS modeling.

For ground spray applications, buffer strips were modeled with AgDrift in tier 1 Ground Spray mode. The boom height was assumed to be low (0.508 m), the droplet size modeled was ASEA medium to coarse and the 90th percentile data was used. A buffer strip of 25 ft was evaluated, consistent with current label requirements for all remaining uses. The modeled spray drift from AgDrift for the 25 ft buffer was 0.45%.

Crop-Specific Application Parameters

Table 3.2 summarizes the crop-specific management practices for all of the assessed uses of azinphos methyl that were used for modeling, including application rates, number of applications per year, application intervals, buffer widths and resulting spray drift values modeled from AgDRIFT, and the first application date for each crop. The date of first application was developed based on several sources of information including data provided by BEAD and Crop Profiles maintained by the USDA. More detail on the crop profiles and the previous assessments may be found at http://pestdata.ncsu.edu/cropprofiles/cropprofiles.cfm.

Table 3.2 Model inputs for maximum label management practices for uses of azinphos methyl for 2007

Crop	App. Rate (lb/A)	Maximum No. Apps.	Minimum App. Interval	Buffer Width	App. Method (% drift)	App. Date
Apples	1.5	3	7 d	25 ft	air blast (2.8)	May 1
Almonds	2	1	NA	25 ft	air blast (2.8)	March 15
Brussels sprouts	0.75	1	NA	25 ft	ground spray (0.45)	Feb 19
Cherries	0.75	2	14 d	25 ft	air blast (2.8)	May 5
Nursery Stock	1	4	10 d	25 ft	air blast (2.8)	May 1
Pears	1.5	2	7 d	25 ft	air blast (2.8)	May 15
Pistachios	2	1	NA	25 ft	air blast (2.8)	August 1
Walnuts	2	1	NA	25 ft	air blast (2.8)	April 1

Note: For all simulations, IPSCND, the disposition of foliar pesticide residues on foliage at harvest was set to 1 so that the residues are applied to the soil.

3.2.2 Model Inputs

Azinphos methyl environmental fate data used for generating model parameters is listed in **Table 3.3**. The input parameters for PRZM and EXAMS are in **Table 3.4**.

Table 3.3 Environmental fate parameters for azinphos methyl.

Fate Parameter	Value	Source
Molecular Mass	317.32 g $\cdot$mol^{-1}	EFGWB One-Liner
Aerobic Soil Metabolism Rate Constant	2.17 x 10^{-2} d^{-1} (31.8 days)	MRID 29900
Anaerobic Soil Metabolism Rate Constant	1.04x10^{-2} d^{-1} (66.7 days)	MRID 29900
K_d	7.6 L $\cdot$kg-soil^{-1} (sandy loam)	MRID 42959702
Solubility	25.10 mg $\cdot$L^{-1}	EFGWB One-Liner
Vapor Pressure	2.2x10^{-7} torr	EFGWB One-Liner
Acidic Hydrolysis Rate Constant	4.78 L $\cdot$(mol-H^{+})$^{-1}$ $\cdot$d^{-1} (39.4 days)	EFGWB One-Liner
Neutral Hydrolysis Constant	7.83x10^{-4} d^{-1} (37.9 days)	Wilkes *et al., 1979*
Alkaline Hydrolysis Constant	82 L$\cdot$(mol-OH^{+})$^{-1}$ $\cdot$d^{-1} (6.6 days)	Wilkes *et al., 1979*
Aqueous Photolysis Constant	0.217 d^{-1} (3.2 days)	MRID 40297001
Washoff Fraction	0.937	Gunther *et al*., 1977
Foliar Degradation Half-life	9.8 days	see text

Table 3.4 Chemistry input parameters for tier 2 (PRZM & EXAMS) simulation of azinphos methyl.

Input Parameter[1]	Value	Justification	Quality
Molecular weight	317.32 g mol^{-1}	calculated	excellent
Solubility	25.10 mg$\cdot$ L^{-1}	measured	very good
Hydrolysis	39.4 (pH 5) 37.5 (pH 7) 6.6 (pH 9)	adjusted for temperature	excellent
Photolysis	3.19 d	measured	very good
Aerobic Soil Metabolism	95.4 d	single value x 3	fair
Water Column Metabolism	190.8 d	aerobic soil x 2	poor
Sediment Metabolism	381.6 d	water column x 2	poor
Foliar Degradation	9.8 d	UCB90 on 7 values	good
Foliar Washoff Coefficient	0.937 cm^{-1}	point estimate from 1 study	fair
Henry's Law Constant	3.66 x 10^{-6} L atm mol^{-1}	estimated from solubility and vapor pressure	poor
Vapor Pressure	2.2 x 10^{-7} torr	measured	good
Soil Water Partition Coefficient (K_d)	7.6 L $\cdot$kg-soil^{-1}	lowest non-sand K_d	good

1 – Input paramters selected using *Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides, Version 2.3, February 28, 2002*

3.2.3 Results

The aquatic EECs for the various scenarios and application practices are listed in **Table 3.5.** Estimated aquatic exposures are highest for azinphos methyl use on brussel sprouts with peak EEC of 6.8 ppb. The use with the next highest exposure concentration was almonds with peak EEC of 6.3 ppb followed by apples, walnuts, pears, nursery stock, pistachios, and cherries with 5.7 ppb, 4.5 ppb, 4.2 ppb, 4.2 ppb, 3.1 ppb, and 1.9 ppb respectively.

Table 3.5 Aquatic EECs (µg/L) for azinphos methyl use on various California agricultural crops. A 25-foot buffer for permanent water bodies was assumed for all scenarios.

Crop	Model Scenario Information			Peak	4 Day Mean	21 Day Mean	60 Day Mean	90 Day Mean
	App. Method	Drift (%)	Scenario					
Almonds	air blast	2.8	CA almond	6.3	5.9	5.0	3.2	2.4
Pistachios	air blast	2.8	CA almond	3.1	2.9	2.2	1.4	1.1
Walnuts	air blast	2.8	CA almond	4.5	4.2	3.5	2.5	1.9
Apples	air blast	2.8	CA fruit	5.7	5.3	4.3	2.9	2.2
Cherries	air blast	2.8	CA fruit	1.9	1.8	1.4	1.0	0.7
Pears	air blast	2.8	CA fruit	4.2	3.9	3.0	2.0	1.5
Brussels sprouts	ground	0.45	CA vegetable	6.8	6.4	5.2	3.4	2.6
Nursery Stock	air blast	2.8	CA nursery	4.2	3.9	3.3	2.5	2.0

3.2.4 Existing Monitoring Data

A critical step in the process of characterizing EECs is comparing the modeled estimates with available surface water monitoring data. Azinphos methyl has a limited set of surface water monitoring data relevant to the CRLF assessment. Most of this data is non-targeted in nature. Included in this assessment are azinphos methyl data from the USGS NAWQA program (http://water.usgs.gov.nawqa), California Department of Pesticide Regulation (CDPR), the State of Washington, Clean Water Act (CWA) 303d listed impaired water bodies' data, and open literature data. In addition, air monitoring data is summarized that is specific to azinphos methyl.

These monitoring data were characterized in terms of general statistics including number of samples, frequency of detection, maximum concentration, and mean from all detections where that level of detail was available.

3.2.4.1 USGS NAWQA Data

Surface water monitoring data from the United States Geological Survey (USGS) NAWQA program was accessed on February 8, 2007 and all data for the state of California was downloaded. A total of 2,003 water samples were analyzed for azinphos methyl. Of these samples, 137 samples had positive detections of azinphos methyl for a frequency of detection of 6.8%. The maximum concentration detected was 1 ppb in the Spanish Grant Combined Drain near Patterson, California.

The next highest concentration was 0.39 ppb in Orestimba Creek near Crows Landing, California. The majority of the remaining detections (75 samples) were found in Orestimba Creek, which is in a predominantly agricultural setting with nuts and stone fruits. The average concentration of all samples was 0.03 ppb while the average concentration of all detections was 0.06 ppb.

3.2.4.2 California Department of Pesticide Regulation (CPR) Data

Surface water monitoring data was accessed from the California Department of Pesticide regulation (CDPR) on February 8, 2007 and all data with analysis for azinphos methyl were extracted. A total of 2,667 samples were available. Of these samples, azinphos methyl was detected in 23 samples for a frequency of detection of 0.9%. The maximum concentration was 0.83 ppb in Del Puerto Creek in Stanislaus County. Of all samples, 7 detections were in Colusa Basin Drain, 12 detections were in Orestimba Creek, 3 samples in the San Joaquin River at Hills Ferry, and 1 detection was in both Del Puerto Creek and the Merced River.

3.2.4.3 Atmospheric Monitoring Data

Available monitoring data for azinphos methyl in air and rainfall were evaluated to provide context to the evaluation of the extent of action area and estimated concentrations in surface water. Based on the available information, azinphos methyl has not been detected in rainwater or snow. Air monitoring data from CDPR indicates that azinphos methyl was detected in two studies conducted in 1987 and in 1994. In the 1987 study, azinphos methyl was detected in approximately 30% of air samples collected out of a total of 170 samples in Kern County. The maximum concentration detected was 0.109 ug/m^3 (8.4 ppt). In the second study from 1994 in Glenn County, azinphos methyl was detected in 13% of samples out of a total of 55 samples with a maximum concentration of 1.7 ug/m^3 (130 ppt). Given the fact that both Kern and Glenn counties are major agricultural locations and that azinphos methyl has not been detected in any of the studies conducted at higher elevations, coupled with the relatively low volatility of azinphos methyl, these detections are likely reflective of near field (spray drift) exposure and are not indicative of long-range transport.

3.2.4.4 Open Literature Data

Ebbert and Embry (2002) assessed the occurrence, distribution, and transport of pesticides in surface waters in the Yakima River Basin, Washington. Data were collected during 1999–2000 as part of the U.S. Geological Survey National Water-Quality Assessment (NAWQA) Program. Samples were collected at 34 sites located throughout the basin. Twenty pesticides were detected during the study, and azinphos methyl was the most widely detected insecticide, with 64 detections out of 98 samples (65%). Sites with the highest (i.e., greater than 70%) azinphos methyl detection rates were associated with drainage basins in which azinphos methyl was applied only to apples (**Table 3.6**). The maximum detected concentration of azinphos methyl was 0.523 μg/L. (This concentration was qualitatively identified and reported as an estimate (Zaugg *et al.*, 1995)).

Table 3.6 Estimates of azinphos methyl usage and detection in select Washington surface waters in 1999			
Location	**Pounds Applied**	**Detection (%)**	**Primary Uses**
Kittitas Valley	6700	Yes (% not specified)	Apples (89%), other tree fruits (10%), potatoes (1%)
Moxee Drainage Basin	18,500	72	Apples (100%)
Granger Drainage Basin	4900	79	Apples (100%)
Yakima River Basin	294,600	50	Apples (88%), pears (7%), cherries (4%)

3.2.4.5 Impaired Waters–Clean Water Act Section 303(d)

Section 303(d) of the Clean Water Act establishes a process for states to identify waters within its boundaries where implementing technology-based controls are inadequate to achieve water quality standards. There are five water bodies that are listed as impaired under Section 303(d) of the Clean Water Act as a result of azinphos methyl contamination (**Table 3.7**).

Table 3.7 Impaired water bodies linked to azinphos methyl contamination		
State	**Waterbody Name**	**Cycle**
CA	Colusa Basin Drain (Central Valley)	2002
CA	Orestimba Creek–above Kilburn Road (Central Valley)	2002
CA	Orestimba Creek–below Kilburn Road (Central Valley)	2002
OR	Neal Creek	2002
WA	Mission Creek	1998

3.2.5 AgDrift Analysis

In order to assess the potential for effects to the aquatic-phase CRLF beyond the application site, an analysis of spray drift distances was completed using all available tools, including AgDrift, AgDISP, and the Gaussian extension to AgDISP. For azinphos methyl use relative to the CRLF an analysis of the results of the screening level risk assessment indicated that spray drift analysis using the most sensitive endpoints for both terrestrial and aquatic species exceeds the range of the AgDrift model for the Tier I orchard airblast mode, Tier II aerial mode, and Tier III aerial mode (no airblast mode beyond tier I exists and therefore aerial was used as a surrogate for airblast). Subsequently, the AgDISP with the Gaussian extension (for longer range transport) was used to evaluate potential distances beyond which exposures would be expected to be below LOC.

The AgDISP model was run in aerial mode as noted above to mimic the airblast applications of azinphos methyl (AgDISP does not have an airblast mode). The model was run with the following settings beyond the standard default settings.

- 30 gal/acre spray volume rate (limit of the model)
- a 15 ft release height (label specific)

- 10 mph limitation (label specific)
- very fine to fine spectrum (default because label limits only for ground and airblast not likely to limit droplet size), and
- no canopy.

For aquatic resources, the most sensitive endpoint was the freshwater invertebrate (*i.e.*, *Gammarus fasciatus*) with an LC_{50} of 0.16 ppb. For the aquatic buffer distance using the settings above the LOC/RQ ratio is 0.00127 (0.05/39.4). Converting this ratio to a fraction of the application rate in kg/ha and using this value as input into the AgDISP model yields at buffer of 685 feet (no Gaussian extension needed).

A similar analysis was conducted using the most sensitive terrestrial endpoint, the honey bee acute contact LD_{50} of 0.063 µg/bee (or 0.491 ppm[7]). Like the aquatic buffer analysis described above, the LOC was compared to the highest RQ. This ratio for terrestrial invertebrates (0.05/817 = 6.12×10^{-5}) multiplied by the application rate in kg/ha yields an initial average deposition of 1.03×10^{-4} kg/ha. Using this value as input into the AgDISP model exceeds the limits of calculation, but incorporation of the Gaussian extension yields a buffer of 3707 feet.

Given that the greatest buffer distance was 3707 feet for terrestrial invertebrates this value was used to buffer the initial area of concern (Figure 2.d) as the first step in defining the final action area.

3.2.6 Evaluation of Azinphos Methyl Oxon

Azinphos methyl has been documented to degrade to its oxygen analog degradate (hereafter referred to as azinphos methyl oxon) in both treated drinking water and in selected environmental fate studies (aerobic soil metabolism and aqueous photolysis). As such, the potential for azinphos methyl oxon to occur in the environment cannot be precluded. In general, azinphos methyl oxon appears to be transient in environmental fate studies. However, there is some evidence of azinphos methyl oxon occurrence in surface waters in that the oxon was detected six times in the USGS Pilot Reservoir Monitoring Study with a single detection in raw water of 0.263 ppb in Oklahoma (out of 605 samples) with a similar detection of 0.3 ppb in the outfall. Of the additional detections, all four samples (two from New York and two from Missouri) were estimated below the level of quantitation and from within the treatment system suggesting they were due to drinking water treatment processes (*i.e.*, chlorination). In addition, other oxons of organophosphate (OP) pesticides have been documented in high mountain lakes (Aston and Seiber, 1997, LeNoir et al, 1999, Zabik and Seiber, 1993), and thus, atmospheric transport cannot be precluded. There is no evidence of the long-range transport of azinphos methyl, or the formation of azinphos methyl oxon beyond the site of application; however, in order to characterize the potential for transport and formation away from application sites the potential for oxon formation has been assessed.

In order to complete this characterization of expected exposure and risk from the use of azinphos methyl, a screening level approach was implemented that first evaluates the potential for transport of azinphos methyl oxon away from the site of application by runoff. This approach assumes that no more the 5% of the applied azinphos methyl is converted to azinphos methyl oxon based on the

[7] Mayer, D. & C. Johansen. 1990. *Pollinator Protection: A Bee & Pesticide Handbook*.Wicwas Press. Cheshire, Conn. p. 161

maximum amount of azinphos methyl oxon seen in environmental fate studies (aerobic soil metabolism; MRID 00029900). If it is assumed that aquatic exposures of azinphos methyl oxon represent an addition to those modeled for parent only then there would be a 5% increase in exposure. Therefore, assuming conversion of the highest EEC of 6.8 ppb for brussel sprouts would yield an azinphos methyl oxon EEC of approximately 0.34 ppb (1/20th of the parent concentrations predicted by PRZM). This represents the potential for azinphos methyl oxon in surface runoff and is likely a highly conservative estimate.

To date, there is no evidence azinphos methyl oxon formation in air, and thus, no quantitative analysis of the impact of azinphos methyl oxon has been made. Given the low vapor pressure (2.2 x 10^{-7} torr) and Henry's Law Constant (3.66x 10^{-6} atm/mol) and lack of documented occurrence, it is not considered a likely route of exposure; however, it is an uncertainty in this assessment.

3.3 Terrestrial Animal Exposure Assessment

The terrestrial exposure model, T-REX (Version 1.3.1, dated December 7, 2006), is used to estimate exposures and risks to terrestrial animals, including birds, mammals, and terrestrial invertebrates. This model was used to assess the dietary residues of azinphos methyl for all of the assessed uses. Input values on avian and mammalian toxicity as well as chemical application and foliar dissipation half-life data are required to run the model. The model generates estimated environmental concentrations (EECs) and calculates risk quotients (RQs). Specifically, the model provides estimates of upper bound and mean concentrations of chemical residues on the surfaces of different food items that may be sources of dietary exposure to the CRLF in the terrestrial-phase (*e.g.,* small and large insects) and to terrestrial animals, such as small mammals and terrestrial invertebrates that the CRLF may prey upon. The surface residue concentration (ppm) is estimated by multiplying the application rate (pounds active ingredient per acre) by a value specific to each food item. Information regarding the T-REX model can be found in **Appendix B**. Model inputs and estimated terrestrial dietary exposures are provided in **Table 3.8.**

Table 3.8 T-REX model inputs for azinphos methyl; Half-life was assumed to be 9.8 days[1] for all uses

Use	Rate (lbs a.i./A)	Minimum Interval (Days)	Max. No. Apps. Per Year	Upper-bound EECs (ppm)			
				Short Grass	Long Grass	Broadleaf Plants, Small Insects	Fruits, Pods, Seeds, Large Insects
Apples	1.5	7	3[2]	713	327	401	45
Brussels Sprouts	0.75	N/A	1	180	83	101	11
Cherries	0.75	14	2	247	113	139	15
Nursery Stock	1	10	4	445	204	251	28
Nuts (Almonds, Pistachios, Walnuts)	2	N/A	1	480	220	270	30
Pears	1.5	7	2	579	266	326	36

[1] For foliar degradation, 7 foliar half-lives measurements are available (Lindquist and Krueger, 1975; Hoskins, 1962; Pree *et al.*, 1976; Winterlin *et al.*, 1974, McDowell *et al*, 1984). Assuming these values are distributed normally, the value which represents the one tail upper 90% confidence limit of the mean is 9.8 d.
[2] The maximum annual application rate for azinphos methyl use on apples is 4.0 lbs a.i./A. Thus, the third application would only be 1 lb a.i./A -- not 1.5 lb a.i./A.

EECs on food items may be compared directly with dietary toxicity data or converted to an oral dose, as is done for small mammals. For mammals, the residue concentration is converted to daily oral dose based on the fraction of body weight consumed daily as estimated through mammalian allometric relationships. The screening-level risk assessment for azinphos methyl uses upper bound predicted residues as the measure of exposure.

The application rate of azinphos methyl on apples is the highest of all assessed uses; thus, the potential terrestrial exposures are highest for the apple use. It should be emphasized that the use of azinphos methyl on apples was modeled using an application rate of 3 applications 7 days apart at 1.5 lbs. a.i./A (a total of 4.5 lbs. a.i./A per year). Since the label specifies a maximum of 4 lbs. a.i./A per year, these dietary residues are slightly overestimated.

This terrestrial exposure model assumes that exposure is a direct function of the application rate and that non-target, small mammals are not likely to reduce pesticide exposure by moving out of the contaminated area. Wang et al. (1999) tested this assumption by placing gray-tailed voles into enclosures planted with a mixture of pasture grasses and applying 1.5 kg a.i/ha (1.34 lbs a.i./A) azinphos methyl (Guthion® 2S) in three treatments: full spray (100% of habitat sprayed), half spray (50% habitat sprayed with azinphos methyl; 50% sprayed with water), and control (100% habitat sprayed with water). Forty-four female and three male voles were tracked before and after azinphos methyl applications using radio telemetry. Following treatment, none of the 47 voles moved out of their established home ranges or from contaminated to uncontaminated areas. Home range size and daily movement patterns were not significantly affected by azinphos methyl treatment. Given access to uncontaminated habitat, gray-tailed voles did not move away from contaminated habitat to avoid azinphos methyl exposure. For this ecological risk assessment, it is reasonable to assume that terrestrial wildlife exposure is directly related to the application rate of azinphos methyl.

4. Effects Assessment

This assessment evaluates the potential for azinphos methyl to adversely affect the California Red Legged Frog (CRLF). As previously discussed in Section 2.8, assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the frog itself, as well as indirect effects, such as reduction of the prey base and/or modification of its habitat. Direct effects to the aquatic-phase of the CRLF are based on toxicity information for freshwater fish and aquatic-phase amphibians, while the terrestrial-phase is based on avian toxicity data since birds are generally used as a surrogate for terrestrial-phase amphibians. Given that the frog’s prey items and habitat requirements are dependent on the availability of freshwater fish and invertebrates, small mammals, terrestrial invertebrates, and aquatic and terrestrial plants, toxicity information for these taxa are also discussed. Acute (short-term) and chronic (long-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on azinphos methyl.

As described in the Agency’s Overview Document (U.S. EPA, 2004a), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include aquatic-phase amphibians, freshwater fish, freshwater invertebrates, birds (surrogate for terrestrial-phase amphibians), mammals, and terrestrial invertebrates. The Agency has determined there will be “no effect" from azinphos methyl use to aquatic and terrestrial plants (see **Section 4.1.3** for more discussion).

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (U.S. EPA, 2004). In order to be included in the ECOTOX database, papers must meet the following minimum criteria:

(1) the toxic effects are related to single chemical exposure;
(2) the toxic effects are on an aquatic or terrestrial plant or animal species;
(3) there is a biological effect on live, whole organisms;
(4) a concurrent environmental chemical concentration/dose or application rate is reported; and
(5) there is an explicit duration of exposure.

Data that pass the ECOTOX screen are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered. The degree to which open literature data are quantitatively or qualitatively characterized is dependent on whether the information is relevant to the assessment endpoints (*i.e.*, maintenance of CRLF survival, reproduction, and growth) identified in Section 2.8. For example, endpoints such as behavior modifications are likely to be qualitatively evaluated unless quantitative relationships between modifications and reduction in species survival, reproduction, and/or growth are available.

4.1 Toxicity of Azinphos Methyl to Aquatic Organisms

Table 4.1 summarizes the most sensitive aquatic toxicity endpoints for the CRLF, based on an evaluation of both the submitted studies and the open literature, as previously discussed. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the CRLF is presented below. Additional information is provided in **Appendix C.**

Table 4.1. Aquatic Toxicity Profile for Azinphos Methyl.

Assessment Endpoint	Measures of Ecological Effects	MRID	Study Classification
Direct toxicity to aquatic-phase CRLF	- Fowlers toad (*Bufo fowleri*) LC_{50} = 109 µg a.i./L (Probit slope assumed to be 4.5) - Fowler's toad (*Bufo fowleri*) estimated NOAEC[1] = 16.5 µg a.i./L	40098001 40579601	Supplemental Acceptable
Indirect toxicity to aquatic-phase CRLF (via toxicity to prey items)	- Northern pike acute LC_{50} = 0.36 µg a.i./L (Probit slope assumed to be 4.5) - *Gammarus fasciatus* acute LC_{50} = 0.16 µg a.i./L (Probit slope assumed to be 4.5) - Northern pike estimated NOAEC[1] = 0.055 µg a.i./L - *Gammarus fasciatus* estimated NOAEC[1] = 0.036 µg a.i./L	40098001 40098001 40579601 00073606	Supplemental Supplemental Acceptable Acceptable

[1] Based on the acute-to-chronic ratio.

Acute toxicity to aquatic animals is categorized using the system shown in **Table 4.2** (U.S. EPA, 2004). Toxicity categories for aquatic plants have not been defined. Based on these categories, azinphos methyl is classified as, at most, very highly toxic to freshwater fish and invertebrates and highly toxic to amphibians on an acute exposure basis.

Table 4.2. Categories of Acute Toxicity for Aquatic Animals

LC_{50}	**Toxicity Category**
< 0.1 mg/L	Very highly toxic
0.1- 1 mg/L	Highly toxic
1 - 10 mg/L	Moderately toxic
10 - 100 mg/L	Slightly toxic
> 100 mg/L	Practically non-toxic

4.1.1 Toxicity to Freshwater Vertebrates (Amphibians and Fish)

EPA typically uses fish as a surrogate for aquatic-phase amphibians when amphibian toxicity data are not available (U.S. EPA, 2004). In the case of azinphos methyl, acute toxicity information is available for several aquatic-phase amphibians, and direct acute risk to the aquatic-phase CRLF was assessed using these data. A chronic NOAEC for amphibians was estimated using the acute-to-chronic ratio for rainbow trout since there were no chronic toxicity data available for amphibians.

Freshwater fish toxicity data were used to assess potential indirect effects of azinphos methyl to the CRLF. Effects to freshwater fish resulting from exposure to azinphos methyl may indirectly affect the CRLF via reduction in available food. As discussed in **Section 2.5.3**, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant, 1985).

4.1.1.1 Aquatic-Phase Amphibians: Acute Exposure (Mortality) Studies

Available acute toxicity studies from the open literature suggest that azinphos methyl is, at most, highly toxic to aquatic-phase amphibians (**Table 4.3**). Summaries of these studies can be found in **Appendix C**. Based on the available information, Fowler's toad is the most sensitive of the 11 amphibian species tested, with an LC_{50} of 109 (72–164) µg a.i/L. This endpoint was used to assess direct acute effects of azinphos methyl to the CRLF.

Table 4.3. Acute toxicity of azinphos methyl to aquatic-phase amphibians.					
Test Species	**Life Stage at Test Start**	**Test Chemical**	**Endpoint (µg a.i/L)**	**ECOTOX Ref/ MRID**	**Description of Use in Document**
Fowler's toad *Bufo woodhousii fowleri*	Tadpole (specific age unknown)	Guthion tech.	96-hour LC_{50} = 109	MRID 40098001	QUANTITATIVE
	4-5-week-old tadpoles	Guthion tech.	96-hour TL_{50} = 130	2891	Qualitative
African clawed frog *Xenopus laevis*	Stage 10-11 Embryo	Guthion tech.	96-hour LC_{50} = 10,630	13686	Qualitative
	2-week-old Tadpoles	Guthion tech.	96-hour LC_{50} = 2940	14957	Qualitative
	Stage 10-11 Embryo	Guthion 2S (22% a.i.)	96-hour LC_{50} = 1600	13686	Qualitative
Lowland frog *Rana ridibunda*	20-day-old Tadpoles	Gusathion-M (23% a.i.)	24-hour LC_{50} = 7180	15315	Qualitative
Green frog *Rana clamitan*	Stage 8[a] Embryo	Guthion 50WP (50% a.i.)	96-hour LC_{50} > 5000 13-day LC_{50}= 2610 16-day[b] LC_{50} > 5000	19300	Qualitative
Northern leopard frog *Rana pipiens*	Stage 8[a] Embryo	Guthion 50WP (50% a.i.)	96-hour LC_{50} > 5000	49995	Qualitative
American toad *Bufo americanus*	Stage 8[a] Embryo	Guthion 50WP (50% a.i.)	96-hour LC_{50} > 5000	49995	Qualitative
Pacific treefrog *Pseudacris regilla*	3-week-old Tadpole	Guthion tech.	96-hour LC_{50} > 3600 10-day NOAEC = 980[c] 10-day LOAEC = 3600[c]	19308	Qualitative
	3-week-old Tadpole	Guthion tech.	96-hour LC_{50} = 4140	14957	Qualitative
	5-week-old Tadpole	Guthion 2S (22% a.i.)	96-hour LC_{50} = 1470 10-day NOAEC =70[c] 10-day LOAEC = 170[c]	19308	Qualitative
Western Chorus Frog *Pseudacris triseriata*	Tadpole (specific age unknown)	Guthion tech.	96-hour LC_{50} = 3200	MRID 40098001	Qualitative
Northwestern salamander *Ambystoma gracile*	6-week-old Larvae	Guthion 2S (22% a.i.)	96-hour LC_{50} = 1670 10-day NOAEC = 100[c] 10-day LOAEC = 220[c]	19308	Qualitative
Spotted salamander *Ambystoma maculatum*	8-week-old Larvae	Guthion 2S (22% a.i.)	96-hour LC_{50} = 1900 10-day NOAEC = 30[c] 10-day LOAEC = 110[c]	19308	Qualitative

[a] According to Gosner (1960)
[b] Discontinuous exposure. (After 4 d exposure treatments were replaced with clean pond water; embryos hatched and began feeding in clean conditions. After 7.5 d in clean water (with renewal every second day), treatments were reintroduced for another 4-d exposure).
[c] Growth was significantly affected.

4.1.1.2 Aquatic-Phase Amphibians: Chronic Exposure (Growth, Reproduction) Studies

No chronic toxicity information is available for aquatic-phase amphibians. Using the acute-to-chronic ratio (ACR) approach with rainbow trout data yields an estimated chronic NOAEC of 16.5 μg/L for Fowler's toad, the most sensitive aquatic-phase amphibian (see calculations below).

ACR = Rainbow trout 96-hour LC50 / Rainbow trout NOAEC = 2.9 μg/L / 0.44 μg/L = 6.6

Estimated Fowler's toad NOAEC = Fowler's toad 96-hour LC50 / ACR = 109 μg/L / 6.6 = **16.5 μg/L**

4.1.1.3 Freshwater Fish: Acute Exposure (Mortality) Studies

Available acute toxicity data indicate that azinphos methyl is very highly toxic to most of the tested fish species (**Table 4.4**). On an acute basis, it appears that fish are generally more sensitive to azinphos methyl than aquatic-phase amphibians. A static acute toxicity test (MRID 40098001) revealed that the northern pike was the most sensitive of the fish species tested, with an LC_{50} of 0.36 (0.27-0.48) μg/L. This endpoint was used to assess potential indirect effects to the CRLF via reduction of prey items. Catfish and bullheads seem to be somewhat less sensitive than the other species tested. For some species, multiple tests were conducted at various temperatures and pH, and the toxicity range is provided.

Table 4.4. Acute toxicity of azinphos methyl to freshwater fish.

Species	Purity (% a.i.)	96-h LC_{50} (μg/L)	Toxicity Category	MRID or Ref.	Study Classification
Northern pike *Esox lucius*	TGAI 93	0.36[a]	very highly toxic	40098001	Supplemental
Brook trout *Salvelinus fontinalis*	TGAI 93	1.2	very highly toxic	40098001	Supplemental
Atlantic salmon *Salmo salar*	TGAI 93	1.8-18 (5 tests)[a] 2.1-3.6 (7 tests)	very highly toxic	40098001	Supplemental
Yellow perch *Perca flavescens*	TGAI 93	2.4-40 (13 tests)	very highly toxic	40098001	Supplemental
	Unspecified Degradate	10-33 (days 0-21)	very highly toxic	40098001	Supplemental
Rainbow trout *Oncorhynchus mykiss*	TGAI 93	2.9-7.1 (4 tests)	very highly toxic	40098001 00158231	Supplemental
	Guthion 50WP	8.8 (4.4 a.i)	very highly toxic	EPA Reg. 3125193	Acceptable
	22 Guthion 2S	27.5 (6.2 a.i.)	very highly toxic	00066046	Supplemental
Black crappie *Pomoxis nigromaculatus*	TGAI 93	3	very highly toxic	40098001	Supplemental
Coho salmon *Oncorhynchus kisutch*	TGAI 93	3.2-6.1 (4 tests)	very highly toxic	40098001	Supplemental
Brown trout *Salmo trutta*	TGAI 93	3.5-6.6 (6 tests)	very highly toxic	40098001	Supplemental
Bluegill sunfish *Lepomis macrochirus*	TGAI 93	4.1-34 (7 tests)	very highly toxic	40098001	Supplemental

Table 4.4. Acute toxicity of azinphos methyl to freshwater fish.					
Species	**Purity (% a.i.)**	**96-h LC_{50} (µg/L)**	**Toxicity Category**	**MRID or Ref.**	**Study Classification**
	22 Guthion 2S	40.4 (8.8 a.i.)	very highly toxic	00066046	Supplemental
Largemouth bass *Micropterus salmoides*	TGAI 93	4.8	very highly toxic	40098001	Supplemental
Green sunfish *Lepomis cyanellus*	TGAI 93	52	very highly toxic	40098001	Supplemental
Golden orfe *Leuciscus idus melanotus*	TGAI 93	120	highly toxic	00067596	Supplemental
Fathead minnow *Pimephales promelas*	TGAI 93	148-293 (2 tests)	highly toxic	40098001	Supplemental
Carp *Cyprinus carpio*	TGAI 93	695	highly toxic	40098001	Supplemental
Channel catfish *Ictalarus punctatus*	TGAI 93	3290	moderately toxic	40098001	Supplemental
Black bullhead *Ictalurus melas*	TGAI 93	3500-4810 (3 tests)	moderately toxic	40098001	Supplemental

[a] Yolk-sac fry

4.1.1.4 Freshwater Fish: Chronic Exposure (Growth, Reproduction) Studies

Chronic toxicity data indicate that azinphos methyl affects the growth and reproduction of fish at levels below one part per billion (**Table 4.5**). Rainbow trout were exposed to mean-measured azinphos methyl treatments of 0.051, 0.14, 0.23, 0.44, and 0.98 µg/L for 60 days. Larval survival was reduced by 65% at the LOAEC (0.98 µg/L).

Table 4.5. Chronic toxicity of azinphos methyl to freshwater fish during an early life-stage toxicity test						
Species	**Purity (% a.i.)**	**NOAEC (µg/L)**	**LOAEC (µg/L)**	**Endpoints Affected**	**MRID**	**Study Classification**
Rainbow trout *Oncorhynchus mykiss*	88.8	0.44[a]	0.98	Larval survival, length, and growth at day 60	40579601	Acceptable

[a] NOAEC for behavioral effects (lethargy) is 0.23 µg/L

No chronic toxicity information is available for the most acutely sensitive freshwater fish, the northern pike. Using the acute-to-chronic ratio (ACR) approach with rainbow trout data yields an estimated chronic NOAEC of 0.055 µg/L for the northern pike (see calculations below).

ACR = Rainbow trout 96-hour LC50 / Rainbow trout NOAEC = 2.9 µg/L / 0.44 µg/L = <u>6.6</u>

Estimated Northern pike NOAEC = Northern pike 96-hour LC50 / ACR = 0.36 µg/L / 6.6 = **0.055 µg/L**

4.1.1.5 Freshwater Vertebrates: Sublethal Effects and Additional Open Literature Information

Several studies suggest that very low levels of azinphos methyl and other organophosphates inhibit cholinesterase (ChE) activity in aquatic animals, such as fish and frogs. Ferrari *et al.* (2004) reported that the azinphos methyl IC_{50} (*i.e.*, concentration that produces 50% cholinesterase inhibition) for rainbow trout, is 0.4 (±0.1) µg/L, which is approximately one order of magnitude below the LC_{50}. The IC_{50} for the toad (*Bufo arenarum*) is 5610 (±810) µg/L, which is about half of the LC_{50}. Sublethal ChE inhibition of 70-90% has been observed in fish species as well (Gruber and Munn, 1998; Varó et al., 2003).

The relationship between sublethal ChE inhibition and the ultimate fitness of a given aquatic species is not well understood (Fulton and Key, 2001). However, Beauvais *et al.* (2000) reported that two organophosphate insecticides altered the normal behavior of larval rainbow trout through cholinesterase inhibition. As cholinesterase activity declined, fish swimming speed and distance were significantly reduced. Behavioral responses such as these may result in alteration of predator/prey relationships, reproductive strategies, migration patterns, *etc*. Nevertheless, these effects are difficult to quantify because they are not clearly tied to the assessment endpoints for the CRLF (*i.e.*, survival, growth, and reproduction of individuals). In addition, differences in habitat and behavior of the tested fish species compared with the CRLF suggest that the results may not be readily extrapolated to frog. Furthermore, there is uncertainty associated with extrapolating effects observed in the laboratory to more variable exposures and conditions in the field. Therefore, potential sublethal effects on fish are evaluated qualitatively in this assessment and are not used as part of the quantitative risk characterization consistent with the Overview Document (USEPA 2004) and the US Fish and Wildlife Service review of EPA's methodology for assessing potential risks to listed species (USFWS/NMFS 2004).

4.1.2 Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of azinphos methyl to the CRLF. Effects to freshwater invertebrates resulting from exposure to azinphos methyl may indirectly affect the CRLF via reduction in available food items. As discussed in Section 2.5.3, the main food source for juvenile aquatic- and terrestrial-phase CRLFs is thought to be aquatic and terrestrial invertebrates found along the shoreline and on the water surface, such as larval alderflies, pillbugs, water striders, and particularly the sowbug.

A summary of available acute and chronic freshwater invertebrate data is provided below in Sections 4.1.2.1 through 4.1.2.3.

4.1.2.1 Freshwater Invertebrates: Acute Exposure (Mortality) Studies

Available freshwater invertebrate acute toxicity studies suggest that azinphos methyl is very highly toxic to several tested species (**Table 4.6**). The most sensitive species appears to be a common amphipod, the scud (*Gammarus fasciatus*), which has a 96-hour LC_{50} of 0.16 (0.08-0.32) µg/L. This information suggests that sowbugs, which are purported to be a favorite prey item of the CRLF, is about two orders of magnitude less sensitive to azinphos methyl than the scud.

Table 4.6. Acute toxicity of azinphos methyl to freshwater invertebrates.					
Species	**Purity (% a.i.)**	**48-h LC_{50} (µg/L)**	**Toxicity Category**	**MRID**	**Study Classification**
Scud *Gammarus fasciatus*	TGAI 93	0.16-0.25 (2 tests)	very highly toxic	40098001	Supplemental
Water flea *Daphnia magna*	TGAI 91	1.13	very highly toxic	00068678	Acceptable
	Guthion 50WP	4.8 (2.4 a.i.)	very highly toxic	40301302	Acceptable
Glass shrimp *Palaemonetes kadiakemsis*	TGAI 93	1.2[a]	very highly toxic	40098001	Supplemental
Stonefly *Pteronarcys californica*	TGAI 93	1.9[a]	very highly toxic	40098001	Supplemental
Sowbug *Asellus brevicaudus*	TGAI 93	21[a]	very highly toxic	40098001	Supplemental
Crayfish *Procambarus* sp.	TGAI 93	56[a]	very highly toxic	40098001	Supplemental

[a] 96-h test

4.1.2.2 Freshwater Invertebrates: Chronic Exposure (Growth, Reproduction) Studies

Available chronic toxicity data indicate that azinphos methyl adversely affects growth and reproduction of a common freshwater zooplankton, *Daphnia magna*, at levels below one part per billion. Sublethal effects in freshwater invertebrates and fish are triggered at approximately the same level of azinphos methyl. Daphnids were exposed to five test concentrations (0.070, 0.12, 0.24, 0.42, and 0.97 µg/L) in a 21-day flow-through chronic toxicity study. Survivorship, length, and fecundity (mean number of young per adult per reproductive day) were significantly reduced in the 0.40 and 0.99 µg/L (mean-measured) treatments (**Table 4.7**).

Table 4.7 Chronic toxicity of azinphos methyl to freshwater invertebrates during a life-cycle toxicity test						
Species	**Purity (% a.i.)**	**NOAEC (µg/L)**	**LOAEC (µg/L)**	**Endpoints Affected**	**Study Classification**	**MRID**
Water flea *Daphnia magna*	99.6	0.25	0.40	Adult length, survival, no. young/adult/day	Acceptable	00073606

No chronic toxicity information is available for the most acutely sensitive freshwater invertebrate, the scud (*Gammarus fasciatus*). Using the acute-to-chronic ratio approach with *Daphnia magna* data yields an estimated chronic NOAEC of 0.036 µg/L for the scud (calculations are shown below).

Acute-to-chronic ratio = *Daphnia magna* 48-hour LC50 / *Daphnia magna* NOAEC = 1.13 µg/L / 0.25 µg/L = <u>4.5</u>

Scud NOAEC = Scud 48-hour LC50 / Acute-to-chronic ratio = 0.16 µg/L / 4.5 = **0.036 µg/L**

4.1.3 Toxicity to Aquatic Plants

There are no registrant-submitted aquatic plant toxicity data for azinphos methyl with which to assess the potential for indirect effects to the CRLF via effects on habitat, cover, and/or primary productivity or effects to the primary constituent elements (PCEs) relevant to the aquatic-phase CRLF (see **Tables 2.5 – 2.6**). However, multiple lines of evidence suggest that azinphos methyl poses minimal if any risk to aquatic plants. Azinphos methyl is an organophosphate insecticide that acts by disrupting nervous system function of exposed animals via acetylcholinesterase inhibition. Further, azinphos methyl has a history of being applied to a myriad of agricultural crops (as per the label), with no known incident of adverse phytotoxic effects. (Note: Two 'plant incidents' are listed in the Ecological Incident Information System (EIIS) report (**Appendix G**). Incident #I013587-010 is a report of azinphos methyl drift from one orchard to a neighboring cherry orchard; there was no plant damage. Incident #I013883-033 is a report of damage to apples following azinphos methyl spray; however, it was suspected that the applicator sprayer was contaminated.) There are no plant protection statements (*i.e.*, warnings of potential adverse plant effects) on the label.

Two studies from the open literature provide further corroboration that azinphos methyl poses minimal if any risk to aquatic plants at environmentally relevant concentrations. Van der Heever and Grobbelaar (1997; ECOTOX ref. 19854) assessed the oxygen-production of *Selenastrum capricornutum* and *Chlorella vulgaris* following exposure to various toxicants, including azinphos methyl. Results indicated that a 30-minute azinphos methyl exposure up to 1 mg/L (the highest level tested) had no effect on the oxygen evolution of either algal species. The authors hypothesized that azinphos methyl may by utilized as a phosphate source by algae. In another study, Van der Heever and Grobbelaar (1998; ECOTOX ref. 19800) assessed the effects of various toxicants, including azinphos methyl, on *chlorophyll a* fluorescence of *Selenastrum capricornutum* for up to 4 hours. Results indicated that azinphos methyl had no effect on the *chlorophyll a* fluorescence up to 1 mg/L (the highest level tested).

4.1.4 Freshwater Field Studies

Sierszen and Lozano (1997) studied the effects of a single application of 0.2, 1.0, 4.0, and 20.0 μg/L azinphos methyl on natural zooplankton communities using littoral ecosystem enclosures. Mean-measured concentrations were 1.33, 4.72, and 20.4 μg/L in the 1.0, 4.0, and 20.0 μg/L nominal treatments, respectively. (The 0.2 μg/L nominal treatment was below the LOQ). Zooplankton were sampled 10 times–twice pre-treatment and 8 times post-treatment. Of the three main groups of zooplankton (cladocerans, copepods, and rotifers), cladocerans were most sensitive to azinphos methyl. Cladoceran taxa accounted for 82% of all significant treatment effects on individual taxa. Most of the effects were observed at the 20 μg/L treatment level; however, 8 of the 12 cladoceran taxa were significantly affected at the 4 μg/L treatment. Azinphos methyl exposure did not elicit consistent, adverse effects on copepods, rotifers, or ostracods. Taxon richness (diversity) decreased with increasing azinphos methyl exposure and was significantly different in the 4.0 and 20.0 μg/L treatments. Recovery of populations and communities ranged from one month (at 4.0 μg/L) to longer than 78 days (at 20 μg/L) following a single application of azinphos methyl.

Schulz and Thiere (2002) evaluated the impacts of azinphos methyl on stream macroinvertebrate communities using a combined microcosm and field approach. Stones were collected from the Lourens River (South Africa) from a control site (free of pesticide contamination) upstream of a 400-ha orchard and transferred to outdoor microcosms so that each microcosm had 12 core macroinvertebrate species and approximately 350 individuals. Microcosms were treated with azinphos methyl at 0 (control), 0.2, 1, 5, or 20 µg/L (mean-measured concentrations were < 0.01 (LOQ), 0.2, 1.0, 4.9, and 19.2 µg/L). Survivorship was assessed 6 days after treatment. Microcosms treated with 4.9 and 19.2 µg/L had significantly lower invertebrate densities. Species diversity was significantly lower in the 19.2 µg/L treatment group, which had an average of 9.7 species compared to 14 in the control group. Schulz and Thiere conducted a parallel macroinvertebrate survey at the control site and a contaminated site (downstream of the orchard) on the Lourens River. Species number was similar at both sites, but abundance and diversity were significantly different. Five of the eight species that were affected by azinphos methyl in the microcosm studies occurred at significantly lower densities or were completely absent at the contaminated field site. Of the four species that were unaffected by azinphos methyl in the microcosm studies, all of them occurred at significantly higher densities at the contaminated field site.

To evaluate the potential impacts of pesticide exposure and other abiotic factors on species abundance and diversity in the Lourens River, Thiere and Schulz (2004) surveyed stream macroinvertebrates above and below a 400-ha orchard area. The sampling site above the orchard (LR1) was free of pesticide contamination, and the site 4000 m downstream of the orchard (LR2) received transient peaks of azinphos methyl, chlorpyrifos, malathion, and endosulfan. The two sampling sites were similar in bottom substrate composition and most abiotic factors, except turbidity and pesticide concentration. The macroinvertebrate communities were similar in terms of number of total individuals, but LR1 had significantly more taxa (11.7) compared to LR2 (8.9). Seven out of 17 taxa occurred had a significantly reduced population or were completely absent at LR2. Based on a community indices for water quality bioassessment, LR2 had a less sensitive community structure, indicating poorer water quality compared to LR1. The authors concluded that pesticide exposure and increased turbidity were the most important factors impacting community structure.

4.2 Toxicity of Azinphos Methyl to Terrestrial Organisms

Table 4.8 summarizes the most sensitive terrestrial toxicity endpoints for the CRLF, based on an evaluation of both the submitted studies and the open literature. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the CRLF is presented below.

Table 4.8. Terrestrial Toxicity Profile for Azinphos Methyl.			
Assessment Endpoint	**Measures of Ecological Effects**	**MRID or Ref.**	**Study Classification**
Direct toxicity to terrestrial-phase CRLF	Northern bobwhite quail acute oral LD_{50} = 32 mg a.i./kg Northern bobwhite quail subacute dietary LC_{50} = 488 ppm Mallard duck chronic reproduction NOAEC = 10.5 ppm	40254801 00022923 40844201	Acceptable Acceptable Acceptable
Indirect toxicity to terrestrial-phase CRLF (via toxicity to prey items)	Honeybee acute contact LD_{50} = 0.063 µg a.i./L = 0.491 ppm[c] Lab rat acute oral LD_{50} = 7.8 mg a.i./kg Gray-tailed vole subacute dietary LC_{50} = 406 ppm Lab rat developmental and chronic NOAEC = 5 ppm	05004151 40280101 Eco 40206 40332601	Acceptable Acceptable Supplemental Acceptable

Acute toxicity to terrestrial animals is categorized using the system shown in **Table 4.9** (U.S. EPA, 2004). Toxicity categories for terrestrial plants have not been defined. Based on these categories, azinphos methyl is classified as, at most, highly toxic to birds and very highly toxic to mammals on an acute exposure basis.

Table 4.9 Qualitative descriptors for avian and mammalian acute toxicity		
Toxicity Category	**Oral LD_{50}**	**Dietary LC_{50}**
Very highly toxic	< 10 mg/kg	< 50 ppm
Highly toxic	10 - 50 mg/kg	50 - 500 ppm
Moderately toxic	51 - 500 mg/kg	501 - 1000 ppm
Slightly toxic	501 - 2000 mg/kg	1001 - 5000 ppm
Practically non-toxic	> 2000 mg/kg	> 5000 ppm

4.2.1 Toxicity to Birds

EPA typically uses birds as a surrogate for terrestrial-phase amphibians when amphibian toxicity data are not available (U.S. EPA, 2004). Since there are no terrestrial-phase amphibian data available for azinphos methyl, acute and chronic avian toxicity data were used to assess the potential direct effects to the CRLF.

4.2.1.1 Birds: Acute Exposure (Mortality) Studies

Acute oral toxicity data are available for a number of avian species. These studies indicate that azinphos methyl ranges from moderately to highly toxic to birds (**Table 4.10**). The most sensitive species, the bobwhite quail, has an LD_{50} of 32 (25-41) mg/kg with a probit slope of 8.8. In this study (MRID 40254801), adult (15-week) bobwhite quail were exposed to 5.6, 11.2, 23.0, 45.0, and 90.0 mg a.i./kg bw. The NOAEL for mortality was 11.2 mg/kg. Sublethal effects including ataxia, wing drop, wing spasms, hyporeactivity, immobility, labored breathing, salivation, and convulsion were observed in all treatments except the lowest dose; thus, the NOAEL for clinical signs of toxicity was 5.6 mg/kg.

Table 4.10 Acute oral toxicity of azinphos methyl to birds.					
Species	**Purity (% a.i.)**	**LD_{50} (mg a.i./kg)**	**Toxicity Category**	**Study Classification**	**MRID**
Bobwhite quail *Colinus virginianus*	TGAI 88.8	32	highly toxic	Acceptable	40254801
	TGAI Not specified	33	highly toxic	Supplemental	40605801
	TGAI 90	60	moderately toxic	Supplemental	00160000
Mallard duck *Anas platyrhynchos*	TGAI 90	136	moderately toxic	Supplemental	00160000
Ring-necked pheasant *Phasianus colchicus*	TGAI 90	74.9	moderately toxic	Supplemental	00160000
	Formulation Not specified	283	moderately toxic	Supplemental	00160000
Chukar *Alectoris chukar*	TGAI 90	84.2	moderately toxic	Supplemental	00160000

Bobwhite quail is also the most sensitive avian species on a subacute dietary toxicity basis, with an LC_{50} of 488 (394-601) ppm (**Table 4.11**). Based on this endpoint, azinphos methyl is highly toxic to birds on a subacute dietary basis.

Table 4.11 Subacute dietary toxicity of azinphos methyl to birds.					
Species	**Purity (% a.i.)**	**LC_{50} (ppm)**	**Toxicity Category**	**Study Classification**	**MRID**
Northern bobwhite quail *Colinus virginianus*	TGAI 92	488	highly toxic	Acceptable	00022923
Japanese Quail *Coturnix japonica*	TGAI 92	639	moderately toxic	Supplemental	00022923
Ring-necked pheasant *Phasianus colchicus*	TGAI 92	1821	slightly toxic	Acceptable	00022923
Mallard duck *Anas platyrhynchos*	TGAI 92	1940	slightly toxic	Acceptable	00022923

4.2.1.2 Birds: Chronic Exposure (Growth, Reproduction) Studies

Chronic avian toxicity data are available for two species (**Table 4.12**). The most sensitive species is the mallard duck, with a reproductive NOAEC of 10.5 ppm. This one-generation reproduction study (MRID 40844201) evaluated the chronic dietary toxicity of azinphos methyl to 18-week old mallard ducks at mean-measured treatment concentrations of 10.5, 32.5, and 96.5 ppm. No treatment-related mortalities or clinical signs of toxicity were observed in adults throughout the course of the study. Females in the 32.5 ppm group weighed significantly (approximately 20%) less than their control counterparts.

Table 4.12 Chronic avian toxicity information for azinphos methyl						
Species	**Purity (% a.i.)**	**NOAEC (ppm)**	**LOAEC (ppm)**	**Endpoints Affected**	**Study Classification**	**MRID**
Mallard duck *Anas platyrhynchos*	88.8	10.5	32.5	Female weight gain	Acceptable	40844201
Northern bobwhite quail *Colinus virginianus*	88.8	36.5	87.4	Eggs laid Eggs set Viable embryos Surviving embryos Surviving hatchlings	Acceptable	41056101

4.2.1.3 Birds: Sublethal Effects and Additional Open Literature Data

Burgess *et al.* (1999) investigated the impact of azinphos methyl spray applications in apple orchards on ChE activity of tree swallows (*Tachycineta bicolor*) and Eastern bluebirds (*Sialia sialis*) nesting in the application area. Mean plasma ChE levels in adult tree swallows were significantly inhibited 41% after a second application of azinphos methyl. In nestlings, brain ChE activity post-spray often fell below predicted activity from control siblings. Survivorship appeared not to be compromised as a result of the observed ChE inhibition.

Gill *et al.* (2000) assessed azinphos methyl exposure to American robins in fruit orchards by measuring plasma ChE activities in nestlings before and after spray events and brain ChE in dead nestlings as well as azinphos methyl residues deposited in model nests placed in trees for spray events. After standardizing for age variations, plasma and brain ChE levels in nestlings sampled from 1 to 4 days post exposure were significantly lower than those sampled before spraying. One day after the spray event, plasma and brain ChE levels in nestling robins were significantly inhibited; maximum inhibition for plasma (34.5%) and brain (53.8%) occurred 4 days after exposure. A number of reproductive endpoints were assessed, but only one significant effect was observed–the proportion of nests with unhatched eggs was significantly higher in exposed orchards.

Gill *et al.* (2004) evaluated the effects of azinphos methyl on ChE activity and general health in zebra finches (*Taeniopygia guttata*) that were previously exposed to *p,p'*-DDE (a commonly detected metabolite of DDT). Zebra finches exposed to azinphos methyl exhibited a dose-response increase in brain and plasma ChE inhibition. Maximum brain ChE inhibition (42.9%) was observed at 45.3 mg/kg, the highest dose tested. Birds in this treatment group did not behave abnormally or die. The authors also found that pre-treatment of *p,p'*-DDE followed by azinphos methyl exposure did not change azinphos methyl ChE inhibition. Immunostimulation was observed in birds dosed 1-year previously with *p,p'*-DDE, and anemia was observed when *p,p'*-DDE and azinphos methyl were combined; these effects were not dose-dependent.

4.2.2 Toxicity to Mammals

Mammalian toxicity data were used to assess potential indirect effects of azinphos methyl to the terrestrial-phase CRLF. Effects to small mammals resulting from exposure to azinphos methyl may indirectly affect the CRLF via reduction in available food. As discussed in **Section 2.5.3**, over 50%

of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant, 1985).

A summary of available acute and chronic mammalian data is provided below in Sections 4.2.2.1 through 4.2.2.3.

4.2.2.1 Mammals: Acute Exposure (Mortality) Studies

Acute oral toxicity studies indicate that the most sensitive mammalian species is the laboratory rat, which has an LD_{50} of 7.8 mg/kg (**Table 4.13**) for azinphos methyl. Based on this endpoint, azinphos methyl is categorized as very highly toxic to mammals.

Table 4.13 Acute oral toxicity of azinphos methyl to mammals.

Species	Purity (% a.i.)	LD_{50} (mg/kg)	Toxicity Category	Study Classification	MRID/Reference
Laboratory rat *Rattus norvegicus*	85	7.8	very highly toxic	Acceptable	40280101
House mouse (wild) *Mus musculus*	99.1	10	highly toxic	Supplemental	Eco 40206
Laboratory mouse *Mus musculus*	99.1	11	highly toxic	Supplemental	Eco 40206
Gray-tailed vole *Microtus canicaudus*	99.1	32	highly toxic	Supplemental	Eco 40206
Deer mouse *Peromyscus maniculatus*	99.1	48	highly toxic	Supplemental	Eco 40206

Subacute dietary toxicity data are available for three mammalian species (**Table 4.14**). These data suggest that azinphos methyl is highly toxic to mammals on a subacute dietary toxicity basis. The gray-tailed vole is the most sensitive species, with a 5-day LC_{50} of 406 (312-858) ppm (Meyers and Wolff, 1994; ECOTOX ref 40206).

Table 4.14 Subacute dietary toxicity of azinphos methyl to mammals.

Species	Purity (% a.i.)	LC_{50} (ppm)	Slope (SE)	Toxicity Category	Study Classification	MRID/ Reference
Gray-tailed vole *Microtus canicaudus*	99.1	406	1.93 (0.6)	highly toxic	supplemental	Eco 40206
Laboratory mouse *Mus musculus*	99.1	543	2.57 (0.84)	moderately toxic	supplemental	Eco 40206
Deer mouse *Peromyscus maniculatus*	99.1	2425	1.45 (0.35)	slightly toxic	supplemental	Eco 40206
	92	>5000	--	practically non-toxic	supplemental	40858301

4.2.2.2 Mammals: Chronic Exposure (Growth, Reproduction) Studies

Chronic mammalian data are available for one species, the laboratory rat (**Table 4.15**). In a two-generation reproduction study in Wistar rats (MRID 40332601), azinphos methyl (87.2%) was administered at dietary concentrations of 0, 5, 15, or 45 ppm (equivalent to 0.25, 0.75, or 2.25 mg/kg/day). The systemic parental NOAEL was 15 ppm (0.75 mg/kg/day), based upon mortality of dams, decreased body weight for P males and F1 males and females, and clinical signs of toxicity, including poor condition and convulsions, at the systemic LOAEL of 45 ppm (2.25 mg/kg/day). The reproductive (offspring) NOAEL and LOAEL were 5 and 15 ppm (0.25 and 0.75 mg/kg/day), respectively. The LOAEL was based on a reduction in pup viability and lactation indices (death of the offspring between the time periods of postnatal days 0-5 and 5-28) and decreased mean total litter weights at weaning on postnatal Day 28. No cholinesterase measurements were taken for either parental animals or pups.

Table 4.15 Chronic mammalian toxicity information for azinphos methyl

Species	Purity (% a.i.)	NOAEC (ppm)	LOAEC (ppm)	Endpoints Affected	Study Classification	MRID
Laboratory rat *Rattus norvegicus*	87.2	5	15	Pup mortality, viability, litter weight	Acceptable	40332601

4.2.3 Toxicity to Terrestrial Invertebrates

Terrestrial invertebrate toxicity data were used to assess potential indirect effects of azinphos methyl to the terrestrial-phase CRLF. Effects to terrestrial invertebrates resulting from exposure to azinphos methyl may indirectly affect the CRLF via reduction in available food.

4.2.3.1 Terrestrial Invertebrates: Acute Exposure (Mortality) Studies

The use of azinphos methyl on agricultural crops may result in exposure to non-target beneficial insects, such as the honey bee. Given that azinphos methyl acts as an insecticide, it is not surprising that this chemical is highly toxic to beneficial insects as well as pest insects. Acute oral and contact studies suggest that azinphos methyl is highly toxic to honey bees (**Table 4.16**). In addition, a foliar residue study with Guthion 50WP indicates that toxic residues can persist on vegetation for up to 13 days post-treatment. The acute contact honey bee LD_{50} = 0.063 µg/bee (converted to 0.491 ppm based on Mayer and Johansen, 1990) is used to assess potential indirect effects to the terrestrial-phase CRLF.

Table 4.16 Acute toxicity of azinphos methyl to honey bees. (TGAI = Technical Grade Active Ingredient)

Species	Purity (% a.i.)	Test Type	Results	Toxicity Category	Study Classification	MRID
Honey bee *Apis mellifera*	TGAI	acute contact (48-h LD50)	LD_{50} = 0.063 µg/bee	highly toxic	Acceptable	05004151
	TGAI	acute oral (48-h LD50)	LD_{50} = 0.15 µg/bee	highly toxic	Acceptable	05004151
	TGAI (% NR)	acute contact (48-h LD50)	LD_{50} = 0.423 µg/bee	highly toxic	Acceptable	00066220
	Guthion 50 WP	foliar residue (3 lb ai/A)	Residues highly toxic for 4-13 days post-treatment	not applicable	Acceptable	40466301

Additional toxicity data for non-target soil and surface insects and mites are available (**Table 4.17**). Results indicate that azinphos methyl is highly toxic to non-target beneficial insects, including bees, wasps, beetles, and mites.

Table 4.17 Acute toxicity of azinphos methyl to non-target beneficial insects (other than honey bees).				
Species	**Purity (% a.i.)**	**Application Rate**	**Results**	**MRID**
Parasitic wasp *Aphytis melinus*	Guthion 50 WP	380 ppm (on lemons)	Highly toxic	05004003
Predaceous beetles (2 spp.) Parasitic wasps (2 spp.)	Guthion 25 WP	0.0477% a.i. (in honey bait)	Highly toxic	05005640
Predaceous beetles (6 spp.) Predaceous wasps (5 spp.)	Guthion 25 WP	0.5 lb ai/100 gal (on waxed paper)	Highly toxic	05003978
Predaceous mite *Amblyseius hibisci*	Guthion 25 WP	0.5 lb ai/100 gal	Highly toxic	05004148

4.2.4 Toxicity to Terrestrial Plants

There are no terrestrial plant toxicity data for azinphos methyl with which to assess the potential for indirect effects to the aquatic- and terrestrial-phase CRLF via effects to riparian vegetation or effects to the primary constituent elements (PCEs) relevant to the aquatic- and terrestrial-phase CRLF (**Tables 2.5 – 2.6**). However, based on the same rationale as for aquatic plants in section 4.1.3, azinphos methyl has "no effects" on terrestrial plants.

4.2.5 Terrestrial Field Studies

An extensive literature exists regarding the adverse ecological impacts of azinphos methyl on terrestrial wildlife. Field studies conducted in apple orchards in Washington (MRID 41139701) and Michigan (MRID 41195901) suggest that spray azinphos methyl (Guthion 35WP) applications can result in the poisoning of a variety of terrestrial animals, including birds, mammals, and reptiles (**Table 4.18**). In Washington, eight orchards were treated with three 1.5 lb ai/acre applications (Guthion 35% WP applied with airblast sprayers) at 7- to 11-day intervals. Eight orchards in Michigan were treated with four 1.5 lb ai/acre applications at 7-to 10-day intervals. The purpose of the studies was to evaluate potential hazards to wildlife based on mortality, population changes of species present in and around the orchards, and from residue levels on foliage and invertebrates. Effects on wildlife were determined from carcass searches pre- and post-treatment, bird censuses based on line transects, and live-trapping of small mammals. Residues were sampled on apple tree foliage, noncrop foliage within and adjacent to orchards and on a few invertebrates collected within the orchards.

Two casualties were recorded pre-treatment and 27 post-treatment in the eight Michigan orchards. Of the 27 post-treatment mortalities (tabulated below), 14 were considered highly likely to have been treatment related, six were possibly treatment related, and seven were not treatment related. Most carcasses were found within the orchards (38%) or along their perimeter (45%), but 17% were located in adjacent areas outside the orchards. In the Washington study, 173 casualties were recorded, including 59 birds of 14 species, 109 mammals of seven species, and five reptiles of two species. Of these, 162 (94%) were found after treatments began. American robins and California quail accounted for 34% and 20%, respectively, of the total avian casualties. Meadow voles comprised 82% of the mammalian casualties. Only 40 of the 173 casualties were analyzed for tissue residue, and 21 (53%) were considered treatment related based on the detection of residue in carcasses. Additionally, 117 other casualties might have been treatment related, based on the circumstances and/or time frames under which carcasses were found. Only 35 casualties were definitely not treatment related. Of the carcasses recovered, 46% were found along orchard perimeters, 41% in orchard interiors, and 13% in areas adjacent to the orchards.

Table 4.18. Presumed and Suspected Treatment-related Mortalities and Casualties During Field Tests in Apple Orchards

Species	Presumed[1]	Suspected[2]
MICHIGAN		
Birds:		
Indigo bunting (*Passerina cyanea*)	1	
Chipping sparrow (*Spizella passerina*)	1	
Killdeer (*Charadrius vociferus*)		1
Unidentified nestling		1
Mammals:		
Northern short-tailed shrew (*Blarina brevicauda*)	4	
Deer mouse (*Peromyscus maniculatus*)	3	
Meadow vole (*Microtus pennsylvanicus*)	2	
Meadow jumping mouse (*Zapus hudsonius*)	1	
Bat	1	
Eastern chipmunk (*Tamias striatus*)		1
Cottontail rabbit (*Sylvilagus* sp.)		1
Unidentified mammal		2
WASHINGTON		
Birds:		
Robin (*Turdus migratorius*)	4	
Meadowlark (*Sturnella neglecta*)	1	
American goldfinch (*Carduelis tristis*)	1	
Chipping sparrow (*Spizella passerina*)	1	
Starling (*Sturnus vulgaris*)	1	
California quail (*Callipepla californica*)		10
Ring-necked pheasant (*Phasianus colchicus*)		1
Black-billed magpie (*Pica pica*)		2
Pigeon (*Columba livia*)		1
Unidentified birds		7
Mammals:		
Meadow vole (*Microtus pennsylvanicus*)	12	
Pocket gopher	1	
Ground squirrel		1
Mountain cottontail (*Sylvilagus* sp.)		1
Muskrat (*Ondatra zibethicus*)		1
Mouse		1
Unidentified mammals		9

[1] azinphos methyl residue detected in carcasses, or impaired animal observed with symptoms typical of cholinesterase poisoning
[2] intoxication suspected based on locations of scavenged carcasses or feather or fur spots and when found in relation to treatment times

Edge et al. (1996) studied the effects of azinphos methyl applications on gray-tailed voles (*Microtus canicaudus*) and deer mice (*Peromyscus maniculatus*) were in 0.2-ha alfalfa enclosures in Oregon. In one study, voles were exposed to a single ground-spray application of either 0, 0.7, 1.4, 2.8, or 4.2 lb ai/acre. Population levels in the 1.4 to 4.2 lb ai/acre enclosures were depressed for four weeks after application. Application at 0.7 lb ai/acre caused little or no detectible demographic responses. In another study, an application of 3.25 lb ai/acre reduced population density and growth, survival, recruitment, and body growth of voles (Schauber et al. 1997). Vole densities were only 40% of the controls and remained depressed for $\geq$ 6 weeks after the single spray application. Deer mouse densities in mowed enclosures also decreased 47% within five days after spraying. Analysis of deer mouse feces indicated that consumption of arthropods just after spraying was greater in treated enclosures than in untreated enclosures, indicating that the mice were eating dead or dying arthropods. A third study found that three applications of 1.45 lb ai/acre applied at 14-day intervals caused significant but short-term reductions in vole survival (Peterson 1996). In that study, effects on survival occurred immediately after application but did not persist for more than a week or two.

Matz *et al.* (1998) compared avian toxicity results from a controlled field study to those from a dietary toxicity laboratory test. In the field study, 12-day old northern bobwhite quail were enclosed in alfalfa fields and exposed to spray applications of azinphos methyl at 0 (control), 0.77, and 3.11 kg a.i./ha (equivalent to 0.69 and 2.75 lb a.i./A). Chick survival was significantly reduced in the 3.11 kg a.i./ha treatment group up to 5 days postspray and at both application rates from 6 to 10 days postspray ($p < 0.05$). Brain acetylcholinesterase (AChE) activity, growth, and weight of crop contents (measure of food consumption) were significantly lower at both treatment concentrations. Based on the Kenaga nomogram employed by OPP to estimate terrestrial exposures, the authors performed a 5-day laboratory dietary toxicity test with 10-day old northern bobwhite quail using equivalent azinphos methyl treatments: 0, 150 (equivalent to 0.77 kg a.i./A), 240, 380, and 600 (equivalent to 3.11 kg a.i./A) ppm. Survivorship was significantly lower for chicks exposed to 600 ppm, and brain AChE and growth were significantly reduced at all azinphos methyl concentrations. Chick survival, brain AChE, and growth in the field were significantly lower compared to equivalent exposures in the laboratory due to differences in exposure routes (*i.e.* inhalation, dermal), behavioral responses, spatial/temporal variability, and indirect effects.

4.3 Use of Probit Slope Response Relationship to Provide Information on the Endangered Species Levels of Concern

The Agency uses the probit dose response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (U.S. EPA, 2004). As part of the risk characterization, an interpretation of acute RQ for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (*i.e.*, mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to azinphos methyl on par with the acute toxicity endpoint selected for RQ calculation. To accomplish this interpretation, the Agency uses the slope of the dose response relationship available from the toxicity study used to establish the acute toxicity measures of effect for each taxonomic group that is relevant to this assessment. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose response relationship. In addition to a single effects probability estimate based on the mean, upper and lower estimates of the effects probability are also provided to account for variance in the slope, if available. The upper and lower bounds of the effects probability are based on available information on the 95% confidence interval of the slope. A statement regarding the confidence in the estimated event probabilities is also included. Studies with good probit fit characteristics (*i.e.*, statistically appropriate for the data set) are associated with a high degree of confidence. Conversely, a low degree of confidence is associated with data from studies that do not statistically support a probit dose response relationship. In addition, confidence in the data set may be reduced by high variance in the slope (i.e., large 95% confidence intervals), despite good probit fit characteristics. In the event that dose response information is not available to estimate a slope, a default slope assumption of 4.5 (lower and upper bounds of 2 to 9) (Urban and Cook, 1986) is used.

Individual effect probabilities are calculated using an Excel spreadsheet tool IECV1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA, OPP, Environmental Fate and Effects Division (June 22, 2004). The model allows for such calculations by entering the mean slope estimate (and the 95% confidence bounds of that estimate) as the slope parameter for the spreadsheet. In addition, the acute RQ is entered as the desired threshold. Results of the probit slope analyses are described in Section 5.2.

5. Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations to determine the potential ecological risk from various azinphos methyl use scenarios within the action area and likelihood of direct and indirect effects on the California Red Legged Frog. The risk characterization provides estimation and description of the likelihood of adverse effects; articulates risk assessment assumptions, limitations, and uncertainties; and synthesizes an overall conclusion regarding the effects determination (*i.e.*, “no effect,” “likely to adversely affect,” or “may affect, but not likely to adversely affect”) for the CRLF.

5.1 Risk Estimation

Risk to the aquatic-phase CRLF is estimated by calculating the ratio of exposure to toxicity using 1-in-10 year estimated environmental concentrations based on the label-recommended usage scenarios for almonds, apples, Brussels sprouts, cherries, nursery stock, pears, pistachios, and walnuts (EECs; **Table 3.5**) and the appropriate toxicity endpoint (see **Table 4.1**). This ratio is the risk quotient (RQ), which is then compared to pre-established acute and chronic levels of concern (LOCs) for each category evaluated (**Appendix D**). In this assessment, 1-in-10 year peak EECs generated by the PRZM/EXAMS models represent acute exposure to the CRLF, freshwater fish, and freshwater invertebrates. The acute endangered species LOC for these taxa is 0.05, and the acute risk LOC is 0.5. Chronic exposures for the CRLF and freshwater fish are represented by the 60-day mean EEC, while chronic exposures for freshwater invertebrates are represented by the 21-day mean EEC. The LOC for chronic exposures to the CRLF, freshwater fish, and freshwater invertebrates is 1.0.

Risk to the terrestrial-phase CRLF is estimated using the T-REX model generated dietary exposure estimates in conservative scenarios to avian species for four forage food types and to mammalian species for five forage food types for azinphos methyl uses in California as described in **Section 3.3**. Risk quotients were calculated using upper-bound EECs for each of these usage scenarios. **Appendix E** provides specific dose- and dietary-based acute and chronic RQs for terrestrial animals (birds, mammals).

Both the dose- and dietary-based acute risk quotients are reported; however, for azinphos methyl, the dose-based RQs are likely a better estimate of actual risk. In general, for pesticides (*i.e.* azinphos methyl) with LD_{50} values less than or equal to 50 mg/kg, the LD_{50} is a better indicator of acute toxicity to birds than the LC_{50} value (Urban 2000). This is due to the inherent uncertainties associated with the subacute dietary tests, in which dose is a function of how much food is consumed. In addition, Matz *et al.* (1998) demonstrated that laboratory dietary toxicity tests for azinphos methyl may underestimate toxic effects because they fail to account for dermal and inhalation exposure and behavioral responses (for study details see **Section 4.2.5**). Thus, for azinphos methyl, dose-based avian acute RQs are preferred over dietary-based.

5.1.1 Direct Effects

Direct effect RQs for the aquatic-phase CRLF are presented in **Table 5.1**. Based on the projected peak 1-in-10 year aquatic EECs (from PRZM/EXAMS) and the available amphibian acute toxicity data, the acute RQs for azinphos methyl uses on almonds, apples, and Brussels sprouts exceed the listed species LOC of 0.05, but are less than the acute risk LOC of 0.5. The acute RQs for all other uses are below the LOC. Based on the projected 60-day mean aquatic EECs and the estimated reproductive NOAEC for frogs (based on the acute-to-chronic ratio for the rainbow trout), none of the chronic RQs exceed the Agency's LOC of 1.0.

Table 5.1. Direct Effect RQs for the Aquatic-Phase California Red Legged Frog Based on 2007 Management Practices

Duration of Exposure	Toxicity Value (µg/L)	Use	EEC (µg/L)[3]	RQ[4]	LOC Exceedance?[5]
Acute	109[1]	Almonds	6.3	0.06	Yes
		Pistachios	3.1	0.03	No
		Walnuts	4.5	0.04	No
		Apples	5.7	0.05	Yes
		Cherries	1.9	0.02	No
		Pears	4.2	0.04	No
		Brussels sprouts	6.8	0.06	Yes
		Nursery Stock	4.2	0.04	No
Chronic	16.5[2]	Almonds	3.2	0.19	No
		Pistachios	1.4	0.08	No
		Walnuts	2.5	0.15	No
		Apples	2.9	0.18	No
		Cherries	1.0	0.06	No
		Pears	2.0	0.12	No
		Brussels sprouts	3.4	0.21	No
		Nursery Stock	2.5	0.15	No

[1] Fowlers toad (*Bufo fowleri*) 96-hour LC_{50} = 109 µg a.i./L (MRID 40098001)
[2] Fowler's toad (*Bufo fowleri*) estimated NOAEC = 16.5 µg a.i./L
[3] EECs are from **Table 3.5**.
[4] RQs for acute exposures utilize peak EECs, while RQs for chronic exposures utilize 60-day EECs.
[5] For acute exposures, the listed species LOC is 0.05. For chronic exposures, the LOC is 1.0.

Direct effect RQs for the terrestrial-phase CRLF were determined using the terrestrial exposure model T-REX to estimate exposures and risks in conservative scenarios to avian and mammalian species for azinphos methyl to all of the assessed uses (see Section 3.3). Risk quotients were calculated using upper-bound EECs for small and large insects (*i.e.*, dietary residues on vegetation were not considered since the CRLF does not consume plants). Avian acute and chronic toxicity data served as a surrogate for the terrestrial-phase CRLF. **Appendix E** provides specific dose- and dietary-based acute and chronic RQs for direct and indirect effects to the terrestrial-phase CRLF.

Based on the T-REX modeled dietary exposures and the surrogate avian toxicity data, the acute and chronic RQs for direct effects to the terrestrial-phase CRLF exceed the acute and chronic LOCs (0.1 and 1.0, respectively) for all of the assessed azinphos methyl uses (**Table 5.2**).

Table 5.2 Direct Effect RQs for the Terrestrial-Phase California Red Legged Frog Based on 2007 Management Practices (Using upper-bound dietary EECs for small and large insects)

Use	Rate (lbs a.i./A)	Number of Apps.	Minimum Interval (Days)	Acute RQ[1]	Acute LOC Exceedance? [2]	Chronic RQ [3]	Chronic LOC Exceedance? [4]
Apples	1.5	3	7	0.32 - 20	Yes	4 - 38	Yes
Brussels Sprouts	0.75	1	NA	< 0.1 - 5	Yes	1 - 10	Yes
Cherries	0.75	2	14	0.11 - 7	Yes	1 - 13	Yes
Nursery Stock	1.0	4	10	0.2 - 13	Yes	3 - 24	Yes
Nuts (Almonds, Pistachios, Walnuts)	2.0	1	NA	0.2 - 14	Yes	3 - 26	Yes
Pears	1.5	2	7	0.3 - 16	Yes	3 - 31	Yes

[1] Based on Northern bobwhite quail acute oral LD_{50} = 32 mg a.i./kg (MRID 40254801) and Northern bobwhite quail subacute dietary LC_{50} = 488 ppm (MRID 00022923). Since the EECs are estimated for several scenarios, the RQs are shown as a range of values. For details, see Appendix E.
[2] Acute listed species LOC = 0.1
[3] Based on mallard duck chronic reproduction NOAEC = 10.5 ppm (MRID 40844201). Since the EECs are estimated for several scenarios, the RQs are shown as a range of values. For details, see Appendix E.
[4] Chronic risk LOC = 1

As stated previously (in Section 3.3), terrestrial exposures for apples were estimated using the application rate of 3 applications 7 days apart at 1.5 lbs a.i./acre. Since the label specifies a maximum of 4 lbs. a.i./A per year, these dietary residues are slightly overestimated. However, if the T-REX model was capable of modeling the actual labeled rate for apples (*i.e.* first 2 applications at 1.5 lbs. a.i./A followed by a third application at 1.0 lbs. a.i./A), the estimated dietary exposures would still be the highest of all of the assessed uses.

5.1.2 Indirect Effects

5.1.2.1 Evaluation of Potential Indirect Effects via Reduction in Food Items (Freshwater Fish)

Indirect effect RQs for the aquatic-phase CRLF via effects to freshwater fish, which are potential prey items, are presented in **Table 5.3**. Based on the projected peak 1-in-10 year aquatic EECs (from PRZM/EXAMS) and acute toxicity data for the most sensitive freshwater fish tested, the acute RQs exceed the acute LOC of 0.5 for all assessed azinphos methyl uses. Likewise, all chronic RQs exceed the LOC of 1.0, based on the projected 60-day mean aquatic EECs and the estimated reproductive NOAEC for the northern pike (based on the acute-to-chronic ratio for the rainbow trout).

Table 5.3. Freshwater Fish RQs Relevant to Indirect Effects to the California Red Legged Frog Based on 2007 Management Practices					
Duration of Exposure	**Toxicity Value (µg/L)**	**Use**	**EEC (µg/L)[3]**	**RQ**	**LOC Exceedance? [4]**
Acute	0.36[1]	Almonds	6.3	18	Yes
		Pistachios	3.1	9	Yes
		Walnuts	4.5	12	Yes
		Apples	5.7	16	Yes
		Cherries	1.9	5.3	Yes
		Pears	4.2	12	Yes
		Brussels sprouts	6.8	19	Yes
		Nursery Stock	4.2	12	Yes
Chronic	0.055[2]	Almonds	3.2	58	Yes
		Pistachios	1.4	25	Yes
		Walnuts	2.5	45	Yes
		Apples	2.9	53	Yes
		Cherries	1.0	18	Yes
		Pears	2.0	36	Yes
		Brussels sprouts	3.4	62	Yes
		Nursery Stock	2.5	45	Yes

[1] Northern pike acute 96-hour LC_{50} = 0.36 µg a.i./L (MRID 40098001)
[2] Northern pike estimated NOAEC = 0.055 µg a.i./L
[3]EECs are from **Table 3.5**. RQs for acute exposures utilize peak EECs, while RQs for chronic exposures utilize 60-day EECs.
[4]For acute exposures, the LOC is 0.05; the acute risk LOC is 0.5. For chronic exposures, the LOC is 1.0.

5.1.2.2 Evaluation of Potential Indirect Effects via Reduction in Food Items (Freshwater Invertebrates)

Table 5.4 presents the RQs for indirect effects to the aquatic-phase CRLF via effects to another potential food source, freshwater invertebrates. Based on the projected peak 1-in-10 year aquatic EECs (from PRZM/EXAMS) and acute toxicity data for the most sensitive freshwater invertebrate tested *(Gammarus fasciatus)*, the acute RQs exceed the acute LOC of 0.5 for all assessed azinphos methyl uses. Likewise, all chronic RQs exceed the LOC of 1.0, based on the projected 21-day mean aquatic EECs and the estimated reproductive NOAEC for the *Gammarus fasciatus* (based on the acute-to-chronic ratio for *Daphnia magna*).

Table 5.4. Freshwater Invertebrate RQs Relevant to Indirect Effects to the California Red Legged Frog Based on 2007 Management Practices					
Duration of Exposure	**Toxicity Value (µg/L)**	**Use**	**EEC (µg/L)[3]**	**RQ**	**LOC Exceedance? [4]**
Acute	0.16[1]	Almonds	6.3	39	Yes
		Pistachios	3.1	19	Yes
		Walnuts	4.5	28	Yes
		Apples	5.7	36	Yes
		Cherries	1.9	12	Yes
		Pears	4.2	26	Yes
		Brussels sprouts	6.8	43	Yes
		Nursery Stock	4.2	26	Yes
Chronic	0.036[2]	Almonds	5.0	139	Yes
		Pistachios	2.2	61	Yes
		Walnuts	3.5	97	Yes
		Apples	4.3	119	Yes
		Cherries	1.4	39	Yes
		Pears	3.0	83	Yes
		Brussels sprouts	5.2	144	Yes
		Nursery Stock	3.3	92	Yes

[1] *Gammarus fasciatus* acute 48-hour LC_{50} = 0.16 µg a.i./L (MRID 40098001)
[2] *Gammarus fasciatus* estimated NOAEC = 0.036 µg a.i./L
[3] EECs are from **Table 3.5**. RQs for acute exposures utilize peak EECs, while RQs for chronic exposures utilize 21-day EECs.
[4] For acute exposures, the listed species LOC is 0.05; the acute risk LOC is 0.5. For chronic exposures, the LOC is 1.0.

5.1.2.3 Evaluation of Potential Indirect Effects via Reduction in Food Items (Small Mammals)

Small mammals are potential prey items for the terrestrial-phase CFLF. Based on the T-REX modeled dietary exposures and mammalian toxicity data, the acute and chronic RQs exceed the acute and chronic LOCs (0.5 and 1.0, respectively) for all of the assessed azinphos methyl uses (**Table 5.5**).

Table 5.5 Indirect Effect RQs for the Terrestrial-Phase California Red Legged Frog Via Direct Effects to Mammals Based on 2007 Management Practices							
Use	**Rate (lbs a.i./A)**	**Number of Apps.**	**Minimum Interval (Days)**	**Acute RQ[1]**	**Acute LOC Exceedance? [2]**	**Chronic RQs[3]**	**Chronic LOC Exceedance? [4]**
Apples	1.5	3	7	0.11 - 40	Yes	9 - 1233	Yes
Blueberries	0.75	2	10	< 0.1 - 15	Yes	3 - 465	Yes
Brussels Sprouts	0.75	1	NA	< 0.1 - 10	Yes	2 - 311	Yes
Cherries	0.75	2	14	< 0.1 - 14	Yes	3 - 427	Yes
Grapes	1	3	14	< 0.1 - 20	Yes	4 - 626	Yes
Nursery Stock	1.0	4	10	< 0.1 - 25	Yes	6 - 770	Yes
Nuts (Almonds, Pistachios, Walnuts)	2.0	1	NA	< 0.1 - 27	Yes	6 - 830	Yes
Parsley	0.5	3	7	< 0.1 - 13	Yes	2 - 411	Yes
Pears	1.5	2	7	< 0.1 - 32	Yes	7 - 1002	Yes

[1] Based on lab rat acute oral LD_{50} = 7.8 mg a.i./kg (MRID 40280101) and gray-tailed vole subacute dietary LC_{50} = 406 ppm (Eco Ref. 40206). Since the EECs are estimated for several scenarios, the RQs are shown as a range of values. For details, see Appendix E.
[2] Acute listed species LOC = 0.1
[3] Based on lab rat developmental and chronic NOAEC = 5 ppm (MRID 40332601). Since the EECs are estimated for several scenarios, the RQs are shown as a range of values. For details, see Appendix E.
[4] Chronic LOC = 1

5.1.2.4 Evaluation of Potential Indirect Effects via Reduction in Food Items (Terrestrial Invertebrates)

Indirect effects to the CRLF as a result of effects to terrestrial invertebrates were assessed by comparing the expected azinphos methyl residues on small and large insects (predicted by the T-REX model) to the acute contact toxicity information for the most sensitive terrestrial invertebrate of the tested species, the honey bee. The RQs exceed the terrestrial invertebrate LOC of 0.05 for all uses, regardless of which EEC (small or large insect) is assumed (**Table 5.6**).

Table 5.6 Indirect Effect RQs for the Terrestrial-Phase California Red Legged Frog Via Direct Effects to Terrestrial Invertebrates Based on 2007 Management Practices							
Use	**Rate (lbs a.i./A)**	**No. Apps.**	**Minimum Interval (Days)**	**Large Insect EEC (ppm)**	**Small Insect EEC (ppm)**	**Large Insect RQ[a, b]**	**Small Insect RQ[a, b]**
Apples	1.5	3	7	45	401	92	817
Brussels Sprouts	0.75	1	NA	11	101	22	206
Cherries	0.75	2	14	15	139	31	283
Nursery Stock	1.0	4	10	28	251	57	511
Nuts (Almonds, Pistachios, Walnuts)	2.0	1	NA	30	270	61	550
Pears	1.5	2	7	36	326	73	664

[a] Based on honey bee LD_{50} = 0.063 µg/bee = 0.491 ppm (Mayer, D. & C. Johansen, 1990)
[b] Acute listed species LOC = 0.05

5.1.3 Primary Constituent Elements of Designated Critical Habitat

5.1.3.1 Aquatic-Phase (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)

Three of the four assessment endpoints for the aquatic-phase primary constituent elements (PCEs) of designated critical habitat for the CRLF are related to potential effects to aquatic and/or terrestrial plants:

- Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.
- Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.
- Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.,* algae)

Due to a lack of phytotoxicity data for azinphos methyl, risk to plants cannot be quantitatively assessed (*i.e.*, RQs are not calculated) for these PCEs. However, as described Sections 4.1.3 and 4.2.4, the Agency has determined that azinphos methyl use has "no effect" on plants.

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." To assess the impact of azinphos methyl on this PCE, acute and chronic freshwater fish and invertebrate toxicity endpoints are used as measures of effects. RQs for these endpoints were calculated in Sections 5.1.2.1 and 5.1.2.2; acute and chronic RQs for freshwater fish and invertebrates exceed the LOCs for all uses.

5.1.3.2 Terrestrial-Phase (Upland Habitat and Dispersal Habitat)

Similar to the aquatic-phase PCEs, three of the four assessment endpoints for the terrestrial-phase PCEs of designated critical habitat for the CRLF are related to potential effects to aquatic and/or terrestrial plants:

- Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance
- Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal
- Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.

Due to a lack of phytotoxicity data for azinphos methyl, risk to plants cannot be quantitatively assessed (*i.e.*, RQs are not calculated) for these PCEs. However, as described Sections 4.1.3 and 4.2.4, the Agency has determined that azinphos methyl use has "no effect" on plants.

The remaining terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of azinphos methyl on this PCE, acute and chronic toxicity endpoints for freshwater fish and invertebrates, birds, mammals, and terrestrial invertebrates are used as measures of effects. RQs for these endpoints were calculated in Sections 5.1.2.1 - 5.1.2.4; RQs for all of these taxa exceed the LOCs for all azinphos methyl uses.

5.2 Risk Description

The risk description synthesizes an overall conclusion regarding the likelihood of adverse impacts leading to an effects determination (*i.e*., "no effect," "may affect, but not likely to adversely affect," or "likely to adversely affect") for the California red legged frog.

If no direct or indirect effect RQs exceed the LOCs (Section 5.1), a "no effect" determination is made for the California red legged frog, based on aziphos methyl's use within the action area. However, if direct or indirect effect LOCs are exceeded, the Agency concludes a preliminary "may affect" determination for the California red legged frog. A summary of the results of the risk estimation (*i.e.*, "no effect" or "may affect" finding) is provided in **Table 5.7** for direct and indirect effects to the CRLF and in **Table 5.8** for the PCEs of designated critical habitat for the CRLF.

Table 5.7. Preliminary Effects Determination Summary for Azinphos Methyl--Direct and Indirect Effects to CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
Aquatic Phase ***(eggs, larvae, tadpoles, juveniles, and adults)***[a]		
Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	May affect	Based on amphibian toxicity data, acute RQs exceed the listed species LOC for almonds, apples, and Brussels sprouts, but do not exceed the acute risk LOC; based on the estimated chronic NOAEC for amphibians, chronic RQs do not exceed the LOC (Table 5.1)
Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	May affect	Acute and chronic freshwater fish and invertebrate RQs exceed LOCs for all assessed uses (Tables 5.3 and 5.4)
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	No effect	Azinphos methyl risk to plants assumed to be negligible based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	No effect	Azinphos methyl risk to plants assumed to be negligible based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Terrestrial Phase ***(Juveniles and adults)***		
Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	May affect	Using birds as a surrogate, acute and chronic RQs exceed the LOC for all assessed uses (Table 5.2)
Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial vertebrates, including mammals and terrestrial phase amphibians)	May affect	Acute and chronic RQs for birds and mammals exceed the LOCs for all uses. Acute RQs for terrestrial invertebrates exceed the LOC (Tables 5.2, 5.5, 5.6).
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation)	No effect	Azinphos methyl risk to plants assumed to be negligible based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident

Table 5.8. Preliminary Effects Determination Summary for Azinphos Methyl—PCEs of designated critical habitat for CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	No effect	Azinphos methyl risk to plants assumed to be negligible based on presumed low phytotoxicity, mode of action, and a history of application to various agricultural crops without incident.
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	No effect	Azinphos methyl risk to plants assumed to be negligible based on presumed low phytotoxicity, mode of action, and a history of application to various agricultural crops without incident.
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	May affect	Acute and chronic freshwater fish and invertebrate RQs exceed LOCs for all assessed uses (Tables 5.3 and 5.4)
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (e.g., algae)	No effect	Azinphos methyl risk to plants assumed to be negligible based on presumed low phytotoxicity, mode of action, and a history of application to various agricultural crops without incident.
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)		
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	No effect	Azinphos methyl risk to plants assumed to be negligible based on presumed low phytotoxicity, mode of action, and a history of application to various agricultural crops without incident.
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	No effect	Azinphos methyl risk to plants assumed to be negligible based on presumed low phytotoxicity, mode of action, and a history of application to various agricultural crops without incident.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	May affect	Acute and chronic RQs for birds and mammals exceed the LOCs for all uses (Tables 5.2, 5.5). Acute RQs for terrestrial invertebrates exceed the LOC (Table 5.6).
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	May affect	Acute and chronic freshwater fish and invertebrate RQs exceed LOCs (Tables 5.3 and 5.4). Acute and chronic RQs for birds and mammals exceed the LOCs (Tables 5.2, 5.5). Acute RQs for terrestrial invertebrates exceed the LOC (Table 5.6).

Following a "may affect" determination, additional information is considered to refine the potential for exposure at the predicted levels based on the life history characteristics (*i.e.*, habitat range, feeding preferences, etc.) of the California red legged frog. Based on the best available information, the Agency uses the refined evaluation to distinguish those actions that "may affect, but are not likely to adversely affect" from those actions that are "likely to adversely affect" the California red legged frog.

The criteria used to make determinations that the effects of an action are "not likely to adversely affect" the California red legged frog include the following:

- <u>Significance of Effect</u>: Insignificant effects are those that cannot be meaningfully measured, detected, or evaluated in the context of a level of effect where "take" occurs for even a single individual. "Take" in this context means to harass or harm, defined as the following:
    - Harm includes significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering.
    - Harass is defined as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering.
- <u>Likelihood of the Effect Occurring</u>: Discountable effects are those that are extremely unlikely to occur. For example, use of dose-response information to estimate the likelihood of effects can inform the evaluation of some discountable effects.
    - <u>Adverse Nature of Effect:</u> Effects that are wholly beneficial without any adverse effects are not considered adverse.

A description of the risk and effects determination for each of the established assessment endpoints for the California red legged frog is provided in **Sections 5.2.1 through 5.2.3**.

5.2.1 Direct Effects to the California Red Legged Frog

5.2.1.1 Aquatic-Phase

Acute Risk

Acute toxicity data for 11 amphibian species suggest that azinphos methyl is moderately to highly toxic to these animals (**Table 4.3**). Based on Tier 2 aquatic model estimates and the acute amphibian toxicity data, the acute RQs for some of the assessed azinphos methyl uses (*i.e.*, almonds, apples, and Brussels sprouts) narrowly exceed the acute endangered species LOC of 0.05, but did not exceed the acute risk LOC. To provide additional information, the probability of an individual mortality to the CRLF was calculated using the probit slope analysis described in Section 4.3. A probit slope value for the acute amphibian toxicity test is not available; therefore, the effect probability was calculated using a default slope assumption of 4.5 with lower and upper bounds of 2 and 9 (Urban and Cook, 1986). Based on the default dose response curve slope of 4.5, the corresponding estimated chance of an individual acute mortality to the aquatic-phase CRLF at an RQ of 0.062 (the highest calculated RQ, for Brussels sprouts) is 1 in $3.63x10^7$. It is recognized that extrapolation of very low probability events is associated with considerable uncertainty in the resulting estimates. In order to explore the possible bounds to such estimates, the upper and lower default bounds (2 to 9) were used to calculate upper and lower estimates of the effects probability associated with the acute RQ. The respective lower and upper effects probability estimates are 1 in 127 to 1.23×10^{27}. Based on the default dose response curve slope of 4.5 (2 – 9), the corresponding estimated chance of an individual acute mortality to the aquatic-phase CRLF at the LOC (0.05) is 1 in 4.18×10^{8} (1 in 216 to 1.75×10^{31}).

Implications of the Phase-Out

To further explore the potential for direct effects to the aquatic-phase CRLF as a result of azinphos methyl use on almonds, apples, and Brussels sprouts, aquatic exposures were modeled for 2008, the first year of the phase-out. As discussed in Section 2.4.4, there are 10 remaining uses of azinphos methyl, which will be phased out by 2012. The use of azinphos methyl on Brussels sprouts and nursery stock will be phased out in 2007; use on almonds, walnuts, and pistachios will be phased out in 2009; and use on apples/crabapples, blueberries, cherries, pears and parsley will be phased out in 2012[8]. In addition, there will be mandatory reductions of annual application rates, and a substantial increase in buffer distances for permanent water bodies (from 25 to 60 feet for apples, cherries, and pears; from 25 to 300-500 feet for almonds; and from 25 to 500 feet for walnuts and pistachios). Further, starting in 2008, the window of time for azinphos methyl application will be narrower for several uses; azinphos methyl may only be applied to almonds and walnuts between June and August, to pistachios between June and October, and to cherries between fruit harvest and leaf fall. As with the previous aquatic exposure estimates, the spray drift buffers were evaluated using AgDrift to determine the appropriate spray drift values for each scenario. Model input values used in this analysis are presented in **Table 5.9**.

Table 5.9 Model inputs for 2008 maximum label management practices for uses of azinphos methyl

Crop	App. Rate (lb/A)	Maximum No. Apps.	Minimum App. Interval	Buffer Width	App. Method (% drift)	App. Date
Apples	1.5	2	7 d	60	air blast (1.8)	May 1
Almonds	2	1	NA	300*	air blast (0.2)	July 1
Cherries	0.75	2	14 d	60	air blast (1.8)	May 5
Pears	1.5	2	7 d	60	air blast (1.8)	May 15
Pistachios	2	1	NA	500	air blast (0.1)	August 1
Walnuts	2	1	NA	500	air blast (0.1)	July 1

Note: For all simulations, IPSCND, the disposition of foliar pesticide residues on foliage at harvest was set to 1 so that the residues are applied to the soil.
* Buffer will be 300 feet Butte, Colusa, Glenn, Madera, Merced, San Joaquin, Solano, Stanislaus, Sutter, Tehama, Yolo, and Yuba counties; 500 feet in all other CA counties

Model-predicted aquatic exposures for azinphos methyl based on the 2008 management practices are presented in **Table 5.10.** The peak 1-in-10 year EECs range from 0.7 μg/L for almonds to 2.7

[8] Blueberries and parsley were not considered in this assessment since the label restricts azinphos methyl use on these crops in California.

μg/L for apples and pears. Compared to the aquatic EECs based on the current (2007) use practices, the aquatic EECs for the 2008 use practices are reduced by about 40% to 90%, with the greatest reductions for almonds, pistachios, and walnuts. The main driver for these reductions is likely the implementation of the spray drift buffers because in California, where the climate is relatively dry, spray drift is a significant route of exposure.

Table 5.10 Aquatic EECs (μg/L) for azinphos methyl use on various California agricultural crops; Based on 2008 management practices

Crop	Model Scenario Information			Peak	4 Day Mean	21 Day Mean	60 Day Mean	90 Day Mean
	App. Method	Drift (%)	Scenario					
Almonds	air blast	1.8	CA almond	0.7	0.7	0.5	0.3	0.3
Pistachios	air blast	0.2	CA almond	0.4	0.4	0.3	0.2	0.1
Walnuts	air blast	1.8	CA almond	0.7	0.7	0.5	0.3	0.3
Apples	air blast	1.8	CA fruit	2.7	2.5	2.0	1.3	1.0
Cherries	air blast	0.1	CA fruit	1.2	1.2	0.9	0.6	0.5
Pears	air blast	0.1	CA fruit	2.7	2.5	1.9	1.3	1.0

Based the predicted peak EECs for 2008 (**Table 5.10**) and the Fowlers toad (*Bufo fowleri*) 96-hour LC_{50} of 109 μg a.i./L (MRID 40098001), the highest RQ would be 0.02 (2.7 μg a.i./L / 109 μg a.i./L) for the use on apples and pears, which does not exceed the acute listed species LOC (0.05).

Chronic Risk

Chronic RQs for the aquatic-phase CRLF were calculated using an estimated NOAEC based on the acute-to-chronic ratio for the rainbow trout since no chronic amphibian data were available for consideration in this risk assessment (see Section 4.1.1.2 for details). Based on the estimated NOAEC (16.5 μg a.i./L) and the model-predicted 60-day mean EECs, none of the chronic RQs exceed the LOC (**Table 5.1**).

Summary

Based on the modeled aquatic exposures for azinphos methyl assuming the use practices for 2008 (the first year of the phase-out) and the available amphibian toxicity data, none of the acute or chronic RQs exceed the Agency's LOCs. Thus, azinphos methyl may affect, but is not likely to adversely affect the aquatic-phase CRLF via direct acute and chronic (reproductive, growth) effects.

5.2.1.2 Terrestrial-Phase

Acute Risk

Acute and chronic RQs exceed the Agency's LOCs for all of the assessed azinphos methyl uses based on the T-REX modeled dietary residues and avian toxicity data. This suggests that azinphos methyl use in the action area may directly affect the terrestrial-phase CRLF. In an effort to refine the acute dose-based risk estimates, the T-REX model was modified to account for the lower metabolic rate and lower caloric requirement of amphibians (compared to birds) in a model referred to as “T-HERPS” (see **Appendix F**). Acute dose-based RQs were recalculated for small (1 g), medium (37 g), and large (238 g) frogs using this model. RQs for frogs that consume small insects and small mammals exceed the LOC for all of the assessed azinphos methyl uses (almonds, apples, Brussels sprouts, cherries, nursery stock, pistachios, pears, walnuts) (**Table 5.11**).

Table 5.11. Upper Bound Kenaga, Acute Terrestrial Herpetofauna Dose-Based Risk Quotients for Azinphos Methyl									
Size Class (grams)	**Adjusted LD50**	**EECs and RQs**							
		Small Insects		**Large Insects**		**Small Mammals**		**Small Amphibians**	
		EEC	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**
APPLES									
1	32.00	15.59	0.49*	1.73	0.05	N/A	N/A	N/A	N/A
37	32.00	15.32	0.48*	1.70	0.05	25.96	0.81**	0.53	0.02
238	32.00	10.04	0.31*	1.12	0.03	17.02	0.53**	0.35	0.01
BRUSSELS SPROUTS									
1	32.00	3.93	0.12*	0.44	0.01	N/A	N/A	N/A	N/A
37	32.00	3.87	0.12*	0.43	0.01	6.55	0.20*	0.13	0.00
238	32.00	2.53	0.08	0.28	0.01	4.29	0.13*	0.09	0.00
CHERRIES									
1	32.00	5.40	0.17*	0.60	0.02	N/A	N/A	N/A	N/A
37	32.00	5.30	0.17*	0.59	0.02	8.99	0.28*	0.18	0.01
238	32.00	3.48	0.11*	0.39	0.01	5.89	0.18*	0.12	0.00
NURSERY STOCK									
1	32.00	9.73	0.30*	1.08	0.03	N/A	N/A	N/A	N/A
37	32.00	9.57	0.30*	1.06	0.03	16.21	0.51**	0.33	0.01
238	32.00	6.27	0.20*	0.70	0.02	10.63	0.33*	0.22	0.01
NUTS (ALMONDS, PISTACHIOS, WALNUTS)									
1	32.00	10.49	0.33*	1.17	0.04	N/A	N/A	N/A	N/A
37	32.00	10.31	0.32*	1.15	0.04	17.47	0.55**	0.36	0.01
238	32.00	6.76	0.21*	0.75	0.02	11.45	0.36*	0.23	0.01
PEARS									
1	32.00	12.66	0.40*	1.41	0.04	N/A	N/A	N/A	N/A
37	32.00	12.44	0.39*	1.38	0.04	21.09	0.66**	0.43	0.01
238	32.00	8.16	0.25*	0.91	0.03	13.82	0.43*	0.28	0.01
*RQ exceeds endangered species LOC (0.1) ** RQ exceeds acute risk LOC (0.5)									

The probability of an individual mortality to the terrestrial-phase CRLF was calculated using the probit slope analysis described in Section 4.3. The probit slope for the acute toxicity test with Northern bobwhite quail (acute oral LD_{50} = 32 mg a.i./kg; MRID 40254801) was 8.8 (confidence intervals unavailable at this time). The corresponding estimated chance of an individual acute mortality to the terrestrial-phase CRLF at an RQ of 0.81 (the highest calculated acute dose-based RQ, for a 37-g frog consuming a small mammal in the apple scenario) is about 1 in 5. Based on the slope of 8.8, the corresponding estimated chance of an individual acute mortality to the terrestrial-phase CRLF at the LOC (0.10) is 1 in $1.46x10^{18}$.

Field studies in apple orchards have documented the poisoning of a variety of terrestrial animals following exposure to spray applications of azinphos methyl at rates similar to the current label rate (discussed in Section 4.2.5). In Washington (Johnson *et al.* 1989, MRID 41139701), eight orchards were treated with azinphos methyl at the current label application rate. In all, 173 (23 species) birds, mammals, and reptiles were found dead, and 94% of the mortalities were post-treatment. In Michigan (Sheeley *et al.,* 1989, MRID 41195901), eight apple orchards were treated with four 1.5 lb ai/acre applications at 7 to 10-day intervals. Twenty-seven animals were found dead post-treatment.

These studies also show that there is concordance between the predicted residues based on the Kenaga nomogram and actual measured residues in the field. In some cases, measured residues from these studies actually exceed those predicted by the Kenaga nomogram. Residues on apple tree foliage were measured within 24 hours of spray blast applications, and mean-measured residues were 199 (range of 82-393) and 236 (range of 105-476) ppm, for Washington and Michigan, respectively. In Washington, measured residues after the second and third application were 312 ppm (123-564 ppm) and 328 ppm (122-611 ppm), respectively. In Michigan, residues measured after the second and third applications were 429 ppm (111-1499 ppm) and 536 ppm (208-1747 ppm), respectively. Predicted residues based on the Kenaga nomogram range from 45-713 ppm for the so-called "upper-bound" estimate and from 21-253 ppm for the mean estimate. Measured residues on other orchard vegetation averaged 26-47% of those on the apple tree foliage. Insects were sampled 24 to 48 hours after application, but few were found, presumably due to high mortality. However, residues on exposed insects on apple trees likely would be comparable to those on the apple tree foliage immediately after application.

Chronic Risk
Since no terrestrial amphibian toxicity data were available for consideration in this assessment, birds (specifically mallard ducks) were used as a surrogate to estimate the potential risks to the terrestrial-phase CRLF. The chronic toxicity endpoint used to calculate chronic RQs for the terrestrial-phase CRLF was a NOAEC of 10.5 ppm for the mallard duck (MRID 40844201); female ducks weighed approximately 20% less than their control counterparts at the LOAEC of 32.5 ppm. In another avian chronic toxicity study, the NOAEC for reproductive effects (*i.e.*, eggs laid, eggs set, viable embryos, surviving embryos, surviving hatchlings) in bobwhite quail was 36.5 ppm.

Based on the T-REX model predicted dietary residues and the avian chronic toxicity data, the chronic RQs for birds exceeded the chronic risk LOC for all of the assessed uses (see **Table 5.2**). Assuming that birds are an appropriate surrogate for the terrestrial-phase CRLF, if actual environmental exposures approach the levels predicted in the T-REX model reproductive and/or growth effects may occur.

Summary
The results from this screening level risk assessment combined with field studies and reported adverse ecological incidents in terrestrial systems (**Appendix G**) suggest that azinphos methyl use in the action area is <u>may affect and is likely to adversely affect</u> the terrestrial-phase CRLF via direct acute and chronic (growth, reproductive) effects.

5.2.2 Indirect Effects to the California Red Legged Frog

5.2.2.1 Indirect Effects via Reduction in Food Items (Freshwater Fish)

Acute Risk

Screening-level risk assessment typically relies on a selected toxicity endpoint (*i.e.* LC_{50}) for the most sensitive species tested. Thus, acute risk quotients for freshwater fish were calculated using the northern pike 96-hour LC_{50} for azinphos methyl. PRZM/EXAMS predicted 1-in-10 year peak aquatic EECs exceed (by about 5 to 19 times) this acute toxicity endpoint of 96-hour LC_{50} of 0.36 µg/L; acute RQs for all of assessed azinphos methyl uses exceed the freshwater fish LOC.

The probability of an individual mortality to the most sensitive freshwater fish tested, the northern pike, was calculated using the probit slope analysis described in Section 4.3. A probit slope value for the acute Northern pike toxicity test (acute 96-hour LC_{50} = 0.36 µg a.i./L; MRID 40098001) is not available; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with lower and upper bounds of 2 and 9 (Urban and Cook, 1986). Based on the default dose response curve slope of 4.5, the corresponding estimated chance of an individual acute mortality to a northern pike at an RQ of 6.8 (the highest calculated RQ, for Brussels sprouts) approaches 1 in 1. The effects probability estimate for the lower and upper bounds (using a slope of 2 and 9, respectively) approaches 1 in 1. Based on the default dose response curve slope of 4.5 (2 – 9), the corresponding estimated chance of an individual acute mortality to a northern pike at the LOC (0.05) is 1 in $4.18x10^{8}$ (1 in 216 to $1.75x10^{31}$).

In addition to estimating risk for the most sensitive freshwater fish species, the northern pike, risks were estimated for a surrogate salmonid, which is known to have a wide range in California (**Figure 5.a**). Specifically, RQs were calculated for the brook trout acute 96-hour LC_{50} of 1.2 µg/L (MRID 40098001).

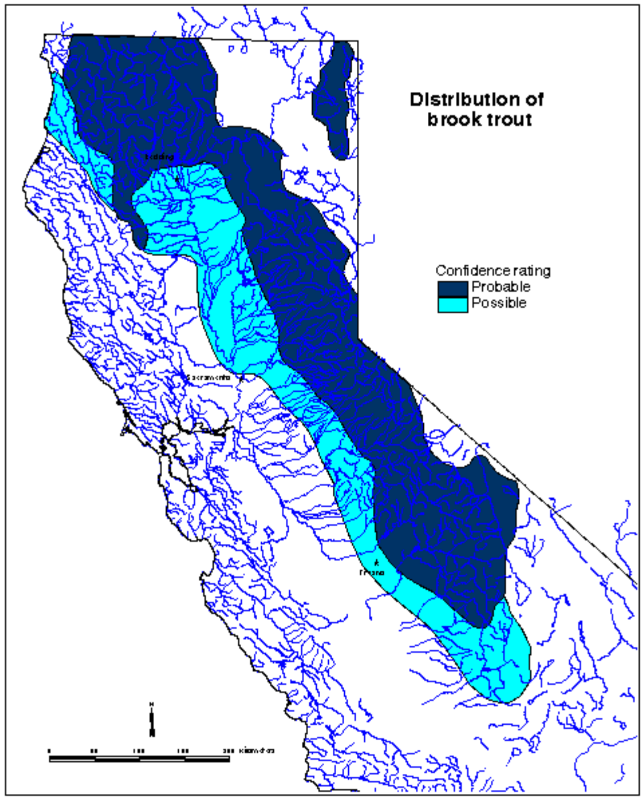


Figure 5.a. Distribution of brook trout in California. Source: University of California-Davis, Information Center for the Environment (http://ice.ucdavis.edu/aquadiv/fishcovs/fishmaps.html

Based on the predicted aquatic EECs and the less sensitive acute toxicity threshold for the brook trout, acute RQs still exceed the acute LOC (0.5) (and the listed species LOC of 0.05) for all of the assessed azinphos methyl uses (**Table 5.12**). Direct effects to freshwater fish may indirectly affect the CRLF via reduced prey availability.

Table 5.12. Freshwater Fish RQs Relevant to Indirect Effects to the California Red Legged Frog for 2007

Duration of Exposure	Toxicity Value (µg/L)	Use	EEC (µg/L)[2]	RQ	LOC Exceedance?[3]
Acute	1.2[1]	Almonds	6.3	5.3	Yes
		Pistachios	3.1	2.6	Yes
		Walnuts	4.5	3.8	Yes
		Apples	5.7	4.8	Yes
		Cherries	1.9	1.6	Yes
		Pears	4.2	3.5	Yes
		Brussels sprouts	6.8	5.7	Yes
		Nursery Stock	4.2	3.5	Yes

[1] Brook trout acute 96-hour LC_{50} = 1.2 µg a.i./L (MRID 40098001)
[2] EECs are from **Table 3.5**.
[3] The acute risk LOC is 0.5.

Assuming the default dose response curve slope of 4.5 for the brook trout, the corresponding estimated chance of an individual acute mortality at an RQ of 5.7 (the highest calculated RQ, for Brussels sprouts) approaches 1 in 1. The effects probability estimates for the lower and upper bounds (using a slope of 2 and 9, respectively) also approach 1 in 1. Based on the default dose response curve slope of 4.5 (2 – 9), the corresponding estimated chance of an individual acute mortality to brook trout at the LOC (0.05) is 1 in 4.18×10^{8} (1 in 216 to 1.75×10^{31}).

Implications of the Phase-Out

To further explore the potential for indirect effects to the aquatic-phase CRLF as a result of azinphos methyl use, aquatic exposures were modeled based on management practices for 2008, the first year of the phase-out (see Section 5.2.1.1). RQs were calculated for both the northern pike and a less sensitive freshwater fish species, the brook trout (**Table 5.13**). Despite the substantial decrease in predicted aquatic exposures, all of the acute RQs exceed the acute risk LOC (0.5), with the exception of the use on walnuts when brook trout toxicity is assumed.

Table 5.13. Freshwater Fish RQs Relevant to Indirect Effects to the California Red Legged Frog Based on 2008 Management Practices

Duration of Exposure	Toxicity Value (µg/L)	Use	EEC (µg/L)[3]	RQ	LOC Exceedance?[4]
Acute	Northern Pike 0.36[1]	Almonds	0.7	2	Yes
		Pistachios	0.4	1	Yes
		Walnuts	0.7	2	Yes
		Apples	2.7	8	Yes
		Cherries	1.2	3	Yes
		Pears	2.7	8	Yes
	Brook Trout 1.2[2]	Almonds	0.7	0.6	Yes
		Pistachios	0.4	0.3	Yes
		Walnuts	0.7	0.6	Yes
		Apples	2.7	2.3	Yes
		Cherries	1.2	1.0	Yes
		Pears	2.7	2.3	Yes

[1] Northern pike acute 96-hour LC_{50} = 0.36 µg a.i./L (MRID 40098001)
[2] Brook trout acute 96-hour LC_{50} = 1.2 µg a.i./L (MRID 40098001)
[3] EECs are from **Table 5.10**.
[4] The acute listed species LOC is 0.05; the acute risk LOC is 0.5.

The presumption of acute risk to freshwater fish is supported by a history of more than 130 adverse ecological incidents in aquatic systems, which have resulted in hundreds of thousands of individual mortalities among aquatic animals, including fish, alligators, turtles, and aquatic snakes (**Appendix G**). All of these reported fish kills are associated with cancelled azinphos methyl uses but one linked to azinphos methyl use on apples (**Table 5.14)**. Taken together, these ecological incidents demonstrate that azinphos methyl can have devastating effects on aquatic ecosystems.

Table 5.14. Adverse Aquatic Incident: Azinphos Methyl						
Use	**EIIS Incident No. (Date)**	**Location**	**Species Affected**	**Magnitude of Effect**	**Incident Summary**	**Certainty Index**
Apples	I004374-006 (01 June 1996)	Jackson, MO	Bluegill Sunfish Fathead Minnow	300 25	Nearby apple orchard was sprayed with azinphos methyl; rain storm led to runoff to affected pond; chemical analyses were not complete at time of report	Probable

Chronic Risk
Chronic toxicity information indicates that reproductive success of the rainbow trout is reduced by about 65% at 1 μg/L. The PRZM/EXAMS modeled 60-day mean EECs based on the 2007 use practices are up to 62 times the estimated NOAEC for reproductive and growth effects in the northern pike. Chronic RQs for freshwater fish exceed the LOC for all of the assessed azinphos methyl uses. Fish that survive initial (peak) exposures may be vulnerable to sublethal effects on normal life processes, such as growth and reproduction.

Implications of the Phase-Out
To further explore the potential for indirect effects to the aquatic-phase CRLF as a result of azinphos methyl use, chronic RQs were calculated for freshwater fish using the predicted aquatic exposures 2008, the first year of the phase-out (see Section 5.2.1.1). Based on these EECs and the northern pike estimated chronic NOAEC all of the chronic RQs exceed the LOC (**Table 5.15**). Aquatic EECs for the use of aziphos methyl on apples and pears are 24 times the estimated NOAEC for chronic effects in northern pike.

Table 5.15. Freshwater Fish RQs Relevant to Indirect Effects to the California Red Legged Frog Based on 2008 Management Practices					
Duration of Exposure	**Toxicity Value (μg/L)**	**Use**	**EEC (μg/L)[2]**	**RQ**	**LOC Exceedance?[3]**
Chronic	0.055[1]	Almonds	0.3	5	Yes
		Pistachios	0.2	4	Yes
		Walnuts	0.3	5	Yes
		Apples	1.3	24	Yes
		Cherries	0.6	11	Yes
		Pears	1.3	24	Yes

[1] Northern pike estimated NOAEC = 0.055 μg a.i./L
[2] EECs are from **Table 5.10**. RQs for acute exposures utilize peak EECs, while RQs for chronic exposures utilize 60-day EECs.
[3] The chronic risk LOC is 1.0.

Summary
Multiple lines of evidence, including the results from this screening level risk assessment, field studies, and reported fish kills associated with azinphos methyl use, suggest that azinphos methyl

use in the action area <u>may affect and is likely to adversely affect</u> the aquatic-phase CRLF indirectly by reducing freshwater fish availability.

5.2.2.2 Indirect Effects via Reduction in Food Items (Freshwater Invertebrates)

Acute Risk

Azinphos methyl is very highly toxic to freshwater invertebrates on an acute basis. RQs calculated using predicted 1-in-10 year peak aquatic EECs and the 48-hour EC_{50} for a common amphipod, the scud (*Gammarus fasciatus*), exceed the acute LOC for all of the assessed uses. The probability of an individual mortality to a scud was calculated using the probit slope analysis described in Section 4.3. A probit slope value for the acute freshwater invertebrate toxicity test is not available; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with lower and upper bounds of 2 and 9 (Urban and Cook, 1986). Based on the default dose response curve slope of 4.5, the corresponding estimated chance of an individual acute mortality to the scud at an RQ of 42.5 (the highest calculated RQ, for Brussels sprouts) approaches 1 in 1. The effects probability estimates for the lower and upper bounds (using a slope of 2 and 9, respectively) also approach 1 in 1. Based on the default dose response curve slope of 4.5 (2 – 9), the corresponding estimated chance of an individual acute mortality to the scud at the LOC (0.05) is 1 in 4.18×10^8 (1 in 216 to 1.75×10^{31}).

As described in Section 2.7, an analysis was completed to assess the potential for risk to aquatic organisms due to downstream transport away from the site of application. This analysis indicates that a total of 194 kilometers of downstream extent is predicted to have exposures above the LOC for freshwater invertebrates. (Lesser distances would be expected for concentrations to be above the LOC for direct effects to the aquatic-phase CRLF and indirect effects via other aquatic taxa (e.g., fish)).

For risk characterization purposes, acute RQs have also been calculated using two less sensitive freshwater invertebrate species, the stonefly (*Pteronarcys californica*) and the sowbug (*Asellus brevicaudus*) (**Table 5.16**). Both of these species are potential prey items for the CRLF; in fact, the sowbug may be a preferred species (Hayes and Tennant, 1985). Based on the stonefly toxicity data, the acute RQs for all of the assessed uses of azinphos methyl exceed the listed species LOC of 0.05 and the acute risk LOC of 0.5. Acute RQs for the sowbug exceed the listed species LOC, but do not exceed the acute risk LOC.

Table 5.16. Freshwater Invertebrate RQs Relevant to Indirect Effects to the California Red Legged Frog For 2007					
Duration of Exposure	**Toxicity Value (µg/L)**	**Use**	**EEC (µg/L)[3]**	**RQ**	**LOC Exceedance?[4]**
Acute	Stonefly 1.9[1]	Almonds	6.3	3.3	Yes
		Pistachios	3.1	1.6	Yes
		Walnuts	4.5	2.4	Yes
		Apples	5.7	3.0	Yes
		Cherries	1.9	1.0	Yes
		Pears	4.2	2.2	Yes
		Brussels sprouts	6.8	3.6	Yes
		Nursery Stock	4.2	2.2	Yes
	Sowbug 21[2]	Almonds	6.3	0.30	Yes
		Pistachios	3.1	0.15	Yes
		Walnuts	4.5	0.21	Yes
		Apples	5.7	0.27	Yes
		Cherries	1.9	0.09	Yes
		Pears	4.2	0.20	Yes
		Brussels sprouts	6.8	0.32	Yes
		Nursery Stock	4.2	0.20	Yes

[1] Stonefly 96-hour LC_{50} = 1.16 µg a.i./L (MRID 40098001)
[2] Sowbug acute 96-hour LC_{50} = 21 µg a.i./L (MRID 40098001)
[3] EECs are from **Table 3.5.**
[4] The acute listed species LOC is 0.05; the acute risk LOC is 0.5.

Implications of the Phase-Out

Acute RQs for freshwater invertebrates were also calculated using the predicted aquatic exposures for 2008 (see Section 5.2.1.1). RQs were calculated using the scud, the water flea, and the sowbug acute toxicity data (**Table 5.17**). Despite the reductions in aquatic EECs, the risk conclusions for the scud remain the same; all RQs exceed the acute risk LOC. For the stonefly, the use of azinphos methyl on apples, cherries, and pears result in RQs above the acute risk LOC while RQs for the nut crops do not. The sowbug is about two orders of magnitude less sensitive than the scud; corresponding RQs fall below the acute risk LOC for all azinphos methyl uses and only exceed the listed species LOC for the apple, cherry, and pear scenarios.

Table 5.17. Freshwater Invertebrate RQs Relevant to Indirect Effects to the California Red Legged Frog Based on 2008 Management Practices					
Duration of Exposure	**Toxicity Value (µg/L)**	**Use**	**EEC (µg/L)[3]**	**RQ**	**LOC Exceedance? [4]**
Acute	Scud 0.16[1]	Almonds	0.7	4	Yes
		Pistachios	0.4	3	Yes
		Walnuts	0.7	4	Yes
		Apples	2.7	17	Yes
		Cherries	1.2	8	Yes
		Pears	2.7	17	Yes
	Stonefly 1.9[2]	Almonds	0.7	0.40	Yes
		Pistachios	0.4	0.21	Yes
		Walnuts	0.7	0.40	Yes
		Apples	2.7	1.4	Yes
		Cherries	1.2	0.63	Yes
		Pears	2.7	1.4	Yes
	Sowbug 21[3]	Almonds	0.7	0.03	No
		Pistachios	0.4	0.02	No
		Walnuts	0.7	0.03	No
		Apples	2.7	0.13	Yes
		Cherries	1.2	0.06	Yes
		Pears	2.7	0.13	Yes

[1] *Gammarus fasciatus* acute 48-hour LC_{50} = 0.16 µg a.i./L (MRID 40098001)
[2] Stonefly 96-hour LC_{50} = 1.16 µg a.i./L (MRID 40098001)
[3] Sowbug acute 96-hour LC_{50} = 21 µg a.i./L (MRID 40098001)
[4] EECs are from **Table 5.10**.
[5] The acute listed species LOC is 0.05; the acute risk LOC is 0.5.

Given the range in sensitivity to azinphos methyl among the tested freshwater invertebrates, the potential risk is greater for some species than others. In general, this risk assessment suggests that azinphos methyl poses acute risk to potential freshwater invertebrate prey for the CRLF. The presumption of acute risk to freshwater invertebrates is supported by a history of adverse ecological incidents in aquatic systems (**Appendix G**). Mortalities among freshwater invertebrates, including grass shrimp, crustaceans, and clams, have been linked to azinphos methyl use. Field studies (*e.g.*, Sierszen and Lozano, 1997) have shown that azinphos methyl can affect zooplankton communities at levels similar to those predicted by PRZM/EXAMS, which may result in a shift toward more tolerant species.

Chronic Risk

Chronic toxicity information for a freshwater zooplankton, *Daphnia magna*, indicates that daphnid survivorship, length, and fecundity (mean number of young per adult per reproductive day) are significantly reduced at 0.40 µg/L azinphos methyl. Using the PRZM/EXAMS 21-day mean aquatic EECs for the 2007 use practices and the estimated NOAEC for the scud (based on *Daphnia magna* toxicity data), chronic RQs exceed the LOC for all assessed azinphos methyl uses. Predicted aquatic exposures are up to 144 times the estimated scud NOAEC for chronic effects.

Implications of the Phase-Out
Chronic RQs for freshwater invertebrates were also calculated using the predicted aquatic exposures for 2008 (see Section 5.2.1.1). RQs were calculated using estimated chronic NOAEC for the scud (**Table 5.18**). Despite the reductions in aquatic EECs, the chronic RQs for all azinphos methyl uses exceed the LOC.

Table 5.18 Freshwater Invertebrate RQs Relevant to Indirect Effects to the California Red Legged Frog Based on 2008 Management Practices

Duration of Exposure	Toxicity Value (μg/L)	Use	EEC (μg/L)[2]	RQ	LOC Exceedance? [3]
Chronic	0.036[1]	Almonds	0.5	14	Yes
		Pistachios	0.3	8	Yes
		Walnuts	0.5	14	Yes
		Apples	2.0	56	Yes
		Cherries	0.9	25	Yes
		Pears	1.9	53	Yes

[1] *Gammarus fasciatus* estimated NOAEC = 0.036 μg a.i./L
[2] EECs are from **Table 5.10**.
[3] The chronic risk LOC is 1.0.

Summary
Multiple lines of evidence, including the results from this screening level risk assessment, field studies, and reported freshwater invertebrate kills associated with azinphos methyl use, suggest that azinphos methyl use in the action area may affect and is likely to adversely affect the aquatic-phase CRLF indirectly by reducing freshwater invertebrate availability through acute and chronic (growth, reproduction) effects.

5.2.2.3 Indirect Effects via Reduction in Food Items (Small Mammals)

Acute Risk
Like other organophosphate insecticides, azinphos methyl exhibits very high acute toxicity in animals by irreversibly inhibiting cholinesterase enzymes, which can lead to a disruption of normal neuromuscular control. To assess the potential indirect effects on the CRLF via impacts on small mammals, dietary residues of azinphos methyl on potential food items were estimated using the T-REX model. Based on these exposure estimates and the available mammalian acute toxicity data, the RQs exceed the acute risk LOC for all of the assessed uses (**Table 5.5**).

The probability of an individual mortality to a small mammal was calculated using the probit slope analysis described in Section 4.3. A probit slope value for the acute lab rat toxicity test (lab rat acute oral LD_{50} = 7.8 mg a.i./kg; MRID 40280101) is not available at this time; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with lower and upper bounds of 2 and 9 (Urban and Cook, 1986). Based on the default dose response curve slope of 4.5, the corresponding estimated chance of an individual acute mortality to a small mammal at an RQ of 40 (the highest calculated RQ, for apples) approaches 100%. The effects probability estimates for the lower and upper bounds (using a slope of 2 and 9, respectively) also approach 100%. Based on the default dose response curve slope of 4.5 (2 – 9), the corresponding estimated chance of an individual acute mortality to a small mammal at the LOC (0.10) is 1 in 2.94×10^5 (1 in 44 to 8.86×10^{18}).

The conclusion of acute risk to small mammals is supported by various field studies and ecological incidents. Terrestrial field and pen studies have documented population-level effects on mammalian species (*i.e.* gray-tailed voles, deer mice) as a result of azinphos methyl exposure in fruit orchards (see Section 4.2.5).

Chronic Risk

Chronic mammalian toxicity data suggest that azinphos methyl can affect mammalian fecundity at relatively low levels. Specifically, in a 2-generation rat reproduction study, the NOAEL and LOAEL were 5 and 15 ppm (0.25 and 0.75 mg/kg/day), respectively. Among the affected endpoints were pup viability and litter weights.

To assess the potential indirect effects on the CRLF via impacts on small mammals, dietary residues of azinphos methyl on potential food items were estimated using the T-REX model. Based on these exposure estimates and the available mammalian chronic toxicity data, the RQs exceed the chronic risk LOC for all of the assessed uses (**Table 5.5**).

Summary

Multiple lines of evidence, including the results from this screening level risk assessment, field studies, and reported incidents of mammalian mortalities associated with azinphos methyl use, suggest that azinphos methyl use in the action area <u>may affect and is likely to adversely affect</u> the terrestrial-phase CRLF indirectly by reducing small mammal availability.

5.2.2.4 Indirect Effects via Reduction in Food Items (Terrestrial Invertebrates)

Acute Risk

Given that azinphos methyl is an organophosphate insecticide, it is not surprising that the chemical exhibits high toxicity to terrestrial invertebrates. The 48-hour acute contact LD_{50} for honey bees is 0.063 µg/bee; any chemical with an acute contact LD_{50} of less than 2 µg/bee is considered “highly toxic.” Acute risk to non-target terrestrial invertebrates was quantitatively estimated in order to evaluate the potential for indirect effects to the CRLF via reduction in terrestrial invertebrate availability. Based on the T-REX predicted azinphos methyl residues on small and large insects and the acute toxicity data for honey bees, acute RQs greatly exceed the LOC for all azinphos methyl uses.

The presumption of acute risk to terrestrial invertebrates is supported by 14 adverse ecological incidents in terrestrial systems in which large numbers of honey bees were killed. Six of these incidents were categorized as possibly associated with the use of azinphos methyl on apples (**Table 5.19**).

Table 5.19 Honey Bee Kills Associated With the Use of Azinphos Methyl on Apples				
EIIS Incident No. (Date)	**Location**	**Magnitude of Effect**	**Incident Summary**	**Certainty Index**
I014341-001 (1996)	Yakima, WA	9 hives	Honey bees onsite of unspecified orchard; chemical residues of azinphos methyl in bees range from 0.5 - 2 ppm	Probable
I014341-003 (1996)	Yakima, WA	430 hives	Honey bees onsite of unspecified orchard; chemical residue of azinphos methyl in bees was 0.23 ppm; two other insecticides were also detected	Possible
I014341-002 (1996)	Yakima, WA	76 hives	Honey bees onsite of unspecified orchard; chemical residue of azinphos methyl in bees was 0.03-0.23 ppm; two other insecticides were also detected	Possible
I014405-028 (03 June 1996)	Yakima, WA	Not reported	Honey bees onsite of unspecified orchard; azinphos methyl detected in bees (levels not reported); orchards were sprayed when weeds were blooming, and honey bees that were attracted to the area were killed	Probable
I013883-032 (1997)	Yakima, WA	20 colonies	Honey bees onsite of unspecified orchard; orchards were sprayed when weeds were blooming, and honey bees that were attracted to the area were killed; no chemical residue analysis reported	Possible
I014341-030 (1999)	Grant, WA	150 hives	Honey bees onsite of unspecified orchard; chemical residues of azinphos methyl in bees ranged from 0.17-18 ppm; one other insecticide was detected at levels up to 1 ppm	Possible

Summary

Based on the results from this screening level risk assessment and a history of honey bee kills, azinphos methyl use in the action area <u>may affect and is likely to adversely affect</u> the aquatic-phase CRLF indirectly by reducing terrestrial invertebrate availability.

5.2.3 Summary of Effects Determinations for the CRLF

Tables 5.20 and 5.21 summarize the direct and indirect effects of azinphos methyl on the CRLF. Based on model predicted environmental exposures and available toxicity information combined with field studies, adverse ecological incidents, pesticide use information in the action area, and information specific to the life history and geographic distribution of the species, azinphos methyl <u>may affect and is likely to adversely affect</u> the CRLF. Specifically, this assessment suggests that azinphos methyl may affect and is likely to adversely affect the CRLF via direct effects to the terrestrial-phase CRLF and indirect effects through reduction of prey items (*i.e.*, freshwater fish, freshwater invertebrates, terrestrial invertebrates, small mammals). Azinphos methyl may affect but is <u>not</u> likely to adversely affect the aquatic-phase CRLF based on use practices for 2008, the first year of the phase-out of the chemical. Further, azinphos methyl has no effect on any assessment endpoints that are based on aquatic or terrestrial plant effects.

Table 5.20 Effects Determination Summary for Direct and Indirect Effects of Azinphos Methyl on the California Red-legged Frog		
Assessment Endpoint	**Effects Determination**	**Basis**
	Aquatic-Phase (Eggs, Larvae, Tadpoles, Adults)	
Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	Not Likely to Adversely Affect	Using acute amphibian toxicity data and an estimated chronic NOAEC (based on fish data), acute RQs for some of the assessed azinphos methyl uses (*i.e.*, almonds, apples, and Brussels sprouts) narrowly exceed the acute endangered species LOC of 0.05. However, if aquatic exposures are modeled assuming the management practices for 2008, the first year of the azinphos methyl phase-out, all acute RQs are below the acute listed species LOC. Chronic RQs do not exceed the LOC based on predicted exposures for 2007 or 2008.
Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	Likely to Adversely Affect	Azinphos methyl acute and chronic RQs exceed LOCs for freshwater fish and invertebrates; risk conclusions supported by field studies and adverse ecological incident reports.
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
	Terrestrial Phase (Juveniles and adults)	
Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	Likely to Adversely Affect	Azinphos methyl acute and chronic RQs exceed LOCs for direct effects using birds as a surrogate; risk conclusions supported by field studies and adverse ecological incident reports.
Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial vertebrates, including mammals and terrestrial phase amphibians)	Likely to Adversely Affect	Azinphos methyl acute and chronic RQs exceed LOCs for terrestrial invertebrates, mammals; risk conclusions supported by field studies and adverse ecological incident reports.
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation)	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.

Table 5.21 Effects Determination Summary for the Critical Habitat Impact Analysis		
Assessment Endpoint	**Effects Determination**	**Basis**
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.[9]	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	Habitat modificaiton	Aquatic invertebrate acute and chronic RQs exceed LOCs; field studies and incident reports support risk conclusions.
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)		
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	No effect	Based on presumed low phytotoxicity, mode of action (acetylcholinesterase inhibition), and a history of application to various agricultural crops without incident.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	Habitat modificaiton	Azinphos methyl poses acute and chronic risk to prey items of the CRLF, including freshwater fish and invertebrates, small mammals, other amphibians, and terrestrial invertebrates.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Habitat modification	Azinphos methyl poses acute and chronic risk to prey items of the CRLF, including freshwater fish and invertebrates, small mammals, other amphibians, and terrestrial invertebrates. Since azinphos methyl poses acute and chronic risk to mammals, the CRLF may be affected via alteration or reduction of refugia in the form of small mammal burrows.

[9] Physico-chemical water quality parameters such as salinity, pH, and hardness are not evaluated because these processes are not biologically mediated and, therefore, are not relevant to the endpoints included in this assessment.

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (i.e., food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (i.e., attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available. Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of CRLF life stages within specific recovery units and/or designated critical habitat within the action area. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the species.
- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual frogs and potential adverse modification to critical habitat.

6. Assumptions, Limitations, Uncertainties, and Data Gaps

6.1 General Exposure

6.1.1 Maximum Use Scenario

The screening-level risk assessment focuses on characterizing potential ecological risks resulting from a maximum use scenario, which is determined from labeled statements of maximum azinphos methyl application rate and number of applications with the shortest time interval between applications. The frequency at which actual uses approach this maximum use scenario may be dependant on insecticide resistance, timing of applications, cultural practices, and market forces.

6.1.2 Azinphos Methyl Oxon

As described in Section 3.2.7, azinphos methyl oxon has been shown to develop in both aerobic soil metabolism and aqueous photolysis studies as well as developing as the result of drinking water treatment (Tierney *et al.*, 2001). In the environmental fate studies, the oxon formed at a maximum of 5% of applied parent under aerobic soil conditions. Assuming the oxon is present at levels that are 5% of the parent azinphos methyl, the aquatic EEC would be approximately 0.34 ppb for the use on Brussels sprouts. This value represents a reasonable approximation of the amount of azinphos methyl oxon that could be found in surface water given that a similar concentration (0.263 ppb) was detected in surface water from a reservoir in Oklahoma in the USGS Pilot Reservoir Monitoring Program (http://md.water.usgs.gov/nawqa/OFR_01-456.pdf). Neither azinphos methyl nor its oxon have been detected in air or rainfall. Volatilization and long-range transport away from the site of application of azinphos methyl is not expected to be a significant route of transport because of the low vapor pressure (2.2 x 10^{-7} torr) and Henry's Law Constant (3.66x 10^{-6} atm/mol) of azinphos methyl.

That said, the potential for increased risk due to oxon formation from azinphos methyl use cannot be precluded. In the cumulative assessment for OP insecticides[10], if there were no toxicity data available for a given OP-oxon, high-end adjustment factors of 10X to 100X were applied to account for the presumed increased toxicity of the oxon relative to the parent chemical. Since there are no toxicity data available for the azinphos methyl oxon, a worst case assumption is that the presence of azinphos methyl oxon in the environment could lead to increased risk relative to the parent by a factor of 5 (assuming a 100X toxicity adjustment relative to the parent and an EEC that is 1/20th of the parent EEC). To explore the potential risk from the oxon, acute RQs for the direct effects to the aquatic-phase CRLF were calculated based on the 2007 and 2008 azinphos methyl use practices (**Table 6.1**), assuming that risk estimates would increase by a factor of 5. For 2007, all of the acute RQs exceed the listed species LOC (0.05) for direct effects to the aquatic-phase CRLF. For 2008, only the azinphos methyl use on apples and pears exceed the acute listed species LOC.

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[10] More detail on the OP cumulative assessment and the characterization of additional risk due to oxon occurrence may be found at http://www.epa.gov/pesticides/cumulative/2006-op/op_cra_appendices_part2.pdf

Table 6.1. Direct Effect RQs for the Aquatic-Phase California Red Legged Frog Based on 2007 Management Practices Assuming the Oxon Increases Risk Estimates By a Factor of 5								
Duration of Exposure	**Oxon Toxicity Adjustment (µg/L)**	**Use**	**2007 Management Practices**			**2008 Management Practices**		
			Oxon EEC Adjustment (µg/L)[2]	**RQ**	**LOC Exceedance? [4]**	**Oxon EEC Adjustment (µg/L)[3]**	**RQ**	**LOC Exceedance? [4]**
Acute	109[1]/100 = 1.09	Almonds	6.3/20 = 0.32	0.29	Yes	0.7/20 = 0.04	0.04	No
		Pistachios	3.1/20 = 0.16	0.14	Yes	0.4/20 = 0.02	0.02	No
		Walnuts	4.5/20 = 0.23	0.21	Yes	0.7/20 = 0.04	0.04	No
		Apples	5.7/20 = 0.29	0.26	Yes	2.7/20 = 0.14	0.13	Yes
		Cherries	1.9/20 = 0.10	0.09	Yes	1.2/20 = 0.06	0.06	No
		Pears	4.2/20 = 0.21	0.20	Yes	2.7/20 = 0.14	0.13	Yes
		Brussels sprouts	6.8/20 = 0.34	0.31	Yes	Use no longer permitted.		
		Nursery Stock	4.2/20 = 0.21	0.20	Yes	Use no longer permitted.		

[1] Fowlers toad (*Bufo fowleri*) 96-hour LC_{50} = 109 µg a.i./L (MRID 40098001)
[2] EECs are from **Table 3.5**.
[3] EECs are from **Table 5.10**.
[4] The acute listed species LOC is 0.05.

6.1.3 Action Area Overlap with Species Range

Appendix A provides an overview of where the action area overlaps with species range as described in Section 2.5.1. The analysis indicates that overlap between action area and species range encompasses 19% of the total species range (defined by critical habitat, core areas, and CNDDB occurrence data) and that overlap occurs in all eight Recovery Units. Specifically, the Recovery Unit with the greatest amount of overlap is Recovery Unit 7 where 31% of the species range is predicted to be exposed to azinphos methyl concentrations above at least one taxon's LOC. Conversely, Recovery Unit 1 has only 4% overlap between action area and species range. A summary of how these overlap ranges were derived along with graphical representations of where the overlap is predicted are presented in Appendix A (Table 3).

Pesticide exposures and predicted risks to the species and its resources (i.e., food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (i.e., attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. That is, areas where overlap occurs between the initial area of concern and the species range are where the risk is presumed to be greatest. Moving from the initial area of concern to the edge of the action area, whether it be defined by spray drift distances or by transport of azinphos methyl downstream from the site of application, the magnitude of exposure decreases as does the potential risk. For example, the action area is defined as extending up to 3707 feet from the site of application based on potential indirect effects to the CRLF via impacts on terrestrial invertebrate prey items. On the other hand, based on potential indirect effects to the CRLF via impacts to aquatic invertebrates, the action area is defined as extending up to 685 feet from the site of application.

By way of comparison, an analysis of overlap between the initial area of concern represented by landcover data for general cropland and orchard lands with species location was completed (Appendix A, Table 4). In this analysis, it is presumed that the highest exposures will occur within the initial area of concern and that exposures will decrease away from the edge of the treated field to a point where exposures are below the LOC. The analysis yields a different picture of the spatial extent of where risk is relative to that of the action area. For example, the total area of overlap for the action area is 19% while the total area of overlap between initial area of concern and species is 4%. Similarly, Recovery Unit 7 which has 31% overlap with action area has only 7% overlap with initial area of concern. Azinphos methyl exposure and associated risks decrease with increasing distance away from the application site, and this analysis shows that risk due to exposures in the treated field will be more spatially focused than those from the entire action area.

6.1.4 CDPR Usage Information

County-level usage data were obtained from California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Four years of data (2002 – 2005) were included in this analysis because statistical methodology for identifying outliers, in terms of area treated and pounds applied, was provided by CDPR for these years only. No methodology for removing outliers was provided by CDPR for 2001 and earlier pesticide data; therefore, this information was not included in the analysis because it may misrepresent actual usage patterns. CDPR PUR documentation indicates that errors in the data may include the following: a misplaced decimal; incorrect measures, area treated, or units; and reports of diluted pesticide concentrations. In addition, it is possible that the data may contain reports for pesticide uses that have been cancelled. The CPDR PUR data does not include home owner applied pesticides; therefore, residential uses are not likely to be reported. As with all pesticide use data, there may be instances of misuse and misreporting. The Agency made use of the most current, verifiable information; in cases where there were discrepancies, the most conservative information was used.

The CDPR PUR data were evaluated to provide some context to the risk conclusions and to the overlap analysis presented in Section 6.1.5. Of the 58 counties in California, 37 counties had reported use of azinphos methyl between 2002 and 2005. This analysis suggests that there are only a few locations where the species range is outside the range of where azinphos methyl has been used. These counties include Alameda, Amador, Calaveras, Marin, Napa, Nevada, Orange, Plumas, and San Francisco counties. Given the size of the action area relative to the intial area of concern (buffered plus downstream extent), the fact that use in a county where it is reported to have occurred in the CDPR PUR data could overlap portions of a county without use (given the buffer of 3707 feet), and uncertainty of the robustness of four years of usage information, it appears that the effects described above cannot be precluded from any of the areas where overlap occurs. The location of the counties with reported azinphos methyl use is presented in **Figure 6a.**

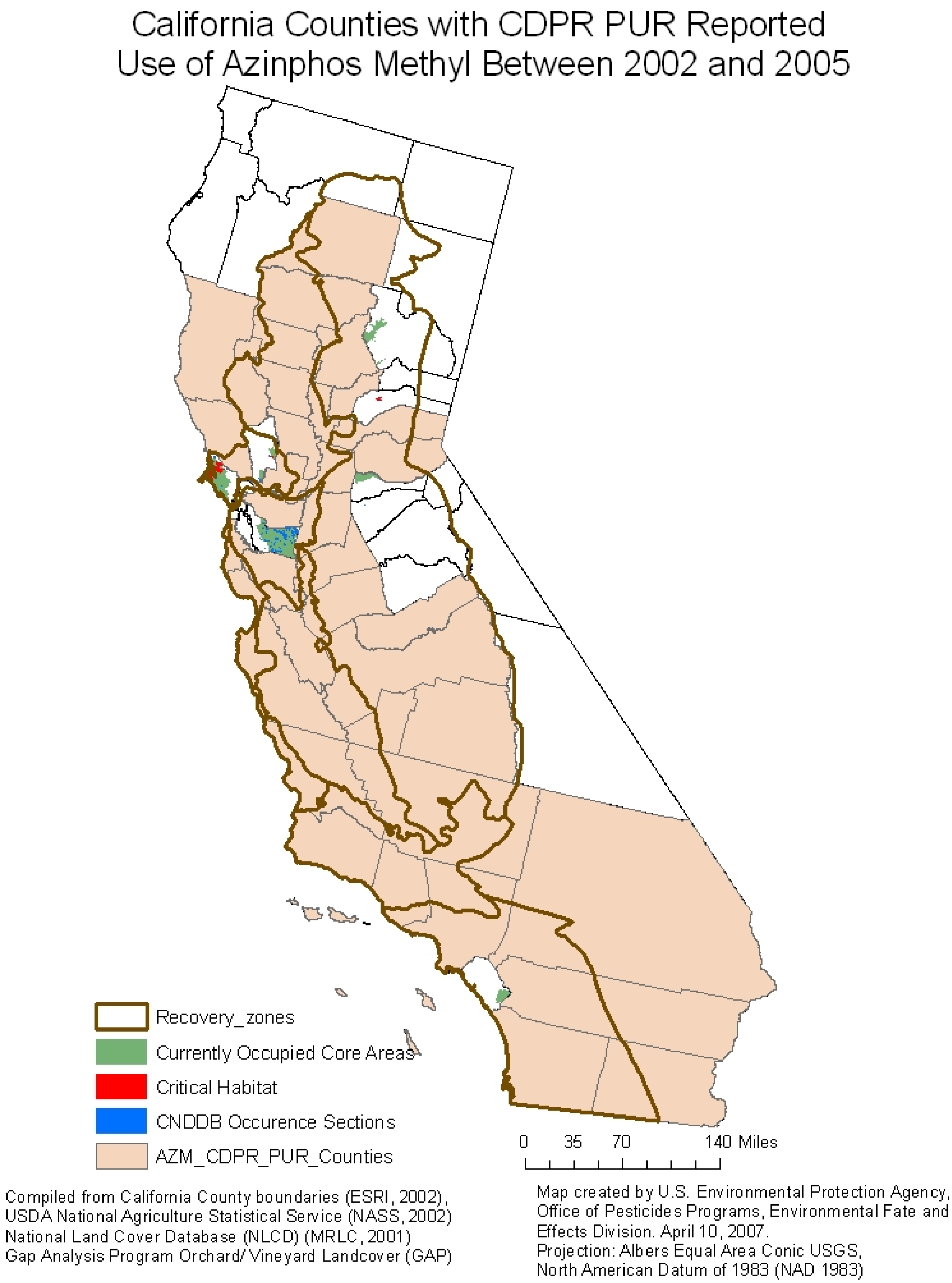

Figure 6.a. Counties with Azinphos Methyl Use Reported Between 2002 and 2005

The usage information from the CDPR PUR database indicates that azinphos methyl has not recently been used in several counties on the three use sites resulting in exceedance of the LOC for direct effects to the aquatic-phase of the CRLF based on the 2007 use practices (almonds, apples, and brussel sprouts); however, that does not necessarily preclude its use in those areas. For example, apples are grown in 5 of the 9 counties where azinphos methyl has not recently been used (*i.e.*, Amador, Calaveras, Marin, Napa, and Nevada). There are only nine California counties where almonds, apples, or Brussels sprouts are not grown (shaded rows in **Table 6.6**).

Of these three crops, by far the most use was reported on almonds with approximately 48,000 lbs annually applied, followed by apples at nearly 18,000 lbs annually and brussel sprouts with 400 lbs annually. During the reporting period azinphos methyl was only applied to brussel sprouts in San Mateo and Santa Cruz counties, while reported use of azinphos methyl was documented in 16 counties on almonds and 28 counties on apples. The county with the highest annual average use of azinphos methyl was Kern County with nearly 25,000 lbs on almonds and 5,000 lbs on apples. Of the remaining counties with reported azinphos methyl use only two counties (San Joaquin and Fresno) had more than 5,000 lbs applied annually.

Table 6.6 Summary of California Counties Where Almonds, Apples, and Brussels Sprouts are Grown[1]
(occurrence in county marked by 'x'; shading indicates none of the three crops are grown in a given county)

County Name	Almond	Apple	Brussels Sprouts
Alameda	x		
Alpine			
Amador		x	
Butte	x	x	
Calaveras		x	
Colusa	x		
Contra Costa	x	x	
Del Norte			
El Dorado	x	x	
Fresno	x	x	
Glenn	x	x	
Humboldt		x	x
Imperial			
Inyo			
Kern	x	x	
Kings	x	x	
Lake	x	x	
Lassen		x	
Los Angeles	x	x	
Madera	x	x	
Marin		x	
Mariposa		x	
Mendocino		x	
Merced	x	x	
Modoc			
Mono			
Monterey		x	
Napa		x	

Table 6.6 Summary of California Counties Where Almonds, Apples, and Brussels Sprouts are Grown[1] (occurrence in county marked by 'x'; shading indicates none of the three crops are grown in a given county)			
County Name	**Almond**	**Apple**	**Brussels Sprouts**
Nevada		x	
Orange		x	
Placer	x	x	
Plumas			
Riverside	x	x	
Sacramento	x	x	
San Benito		x	
San Bernardino	x	x	
San Diego	x	x	
San Francisco			
San Joaquin	x	x	
San Luis Obispo	x	x	
San Mateo		x	x
Santa Barbara	x	x	
Santa Clara	x	x	
Santa Cruz		x	x
Shasta	x	x	
Sierra			
Siskiyou		x	
Solano	x		
Sonoma		x	
Stanislaus	x	x	
Sutter	x	x	
Tehama	x		
Trinity		x	
Tulare	x	x	
Tuolumne		x	
Ventura		x	
Yolo	x	x	
Yuba	x	x	

[1] Source http://www.nass.usda.gov/Census/Create_Census_US_CNTY.jsp

6.2 Aquatic Assessment

6.2.1 Aquatic Exposure Models

In general, the linked PRZM/EXAMS model produces estimated aquatic concentrations that are expected to be exceeded once within a ten-year period. The Pesticide Root Zone Model is a process or "simulation" model that calculates what happens to a pesticide in a farmer's field on a day-to-day basis. It considers factors such as rainfall and plant transpiration of water, as well as how and when the pesticide is applied. It has two major components: hydrology and chemical transport. Water movement is simulated by the use of generalized soil parameters, including field capacity, wilting point, and saturation water content. The chemical transport component can simulate pesticide application on the soil or on the plant foliage. Dissolved, adsorbed, and vapor-phase concentrations in the soil are estimated by simultaneously considering the processes of pesticide uptake by plants, surface runoff, erosion, decay, volatilization, foliar wash-off, advection, dispersion, and retardation.

Uncertainties associated with each of these individual components add to the overall uncertainty of the modeled concentrations. Additionally, model inputs from the environmental fate degradation studies are chosen to represent the upper confidence bound on the mean values that are not expected to be exceeded in the environment approximately 90 percent of the time. Mobility input values are chosen to be representative of conditions in the environment. The natural variation in soils adds to the uncertainty of modeled values. Factors such as application date, crop emergence date, and canopy cover can also affect estimated concentrations, adding to the uncertainty of modeled values. Factors within the ambient environment such as soil temperatures, sunlight intensity, antecedent soil moisture, and surface water temperatures can cause actual aquatic concentrations to differ for the modeled values.

Additionally, the rate at which azinphos methyl is applied and the percent of crops that are actually treated with azinphos methyl may be lower than the Agency's default assumption of the maximum allowable application rate being used and the entire crop being treated. The geometry of a watershed and limited meteorological data sets also add to the uncertainty of estimated aquatic concentrations.

Unlike spray drift, tools are currently not available to evaluate the effectiveness of a vegetative setback on runoff and loadings. The effectiveness of vegetative setbacks is highly dependent on the condition of the vegetative strip. For example, a well-established, healthy vegetative setback can be a very effective means of reducing runoff and erosion from agricultural fields. Alternatively, a setback of poor vegetative quality or a setback that is channelized can be ineffective at reducing loadings. Until such time as a quantitative method to estimate the effect of vegetative setbacks on various conditions on pesticide loadings becomes available, the aquatic exposure predictions are likely to overestimate exposure where healthy vegetative setbacks exist and underestimate exposure where poorly developed, channelized, or bare setbacks exist.

6.2.2 Model Inputs

The standard ecological water body scenario (EXAMS pond) used to calculate potential aquatic exposure to pesticides is intended to represent conservative estimates, and to avoid underestimations of the actual exposure. The standard scenario consists of application to a 10-hectare field bordering a 1-hectare, 2-meter deep (20,000 m3) pond with no outlet. Exposure estimates generated using the EXAMS pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and lower order streams. As a group, there are factors that make these water bodies more or less vulnerable than the EXAMS pond. Static water bodies that have larger ratios of pesticide-treated drainage area to water body volume would be expected to have higher peak EECs than the EXAMS pond. These water bodies will be either smaller in size or have larger drainage areas. Smaller water bodies have limited storage capacity and thus may overflow and carry pesticide in the discharge, whereas the EXAMS pond has no discharge. As watershed size increases beyond 10-hectares, it becomes increasingly unlikely that the entire watershed is planted with a single crop that is all treated simultaneously with the pesticide. Headwater streams can also have peak concentrations higher than the EXAMS pond, but they likely persist for only short periods of time and are then carried and dissipated downstream.

The Agency acknowledges that there are some unique aquatic habitats that are not accurately captured by this modeling scenario and modeling results may, therefore, under- or over-estimate exposure, depending on a number of variables. For example, aquatic-phase CRLFs may inhabit water bodies of different size and depth and/or are located adjacent to larger or smaller drainage areas than the EXAMS pond. The Agency does not currently have sufficient information regarding the hydrology of these aquatic habitats to develop a specific alternate scenario for the CRLF. CRLFs prefer habitat with perennial (present year-round) or near-perennial water and do not frequently inhabit vernal (temporary) pools because conditions in these habitats are generally not suitable (Hayes and Jennings 1988). Therefore, the EXAMS pond is assumed to be representative of exposure to aquatic-phase CRLFs. In addition, the Services agree that the existing EXAMS pond represents the best currently available approach for estimating aquatic exposure to pesticides (USFWS/NMFS 2004).

6.2.3 Potential Aquatic Exposures Relative to CRLF Habitat

The standard ecological water body scenario (EXAMS pond) used to calculate potential aquatic exposure to pesticides is intended to represent conservative estimates, and to avoid underestimations of the actual exposure. The standard scenario consists of application to a 10-hectare field bordering a 1-hectare, 2-meter deep (20,000 m^3) pond with no outlet. Exposure estimates generated using the EXAMS pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and lower order streams. As a group, there are factors that make these water bodies more or less vulnerable than the EXAMS pond. Static water bodies that have larger ratios of pesticide-treated drainage area to water body volume would be expected to have higher peak EECs than the EXAMS pond. These water bodies will be either smaller in size or have larger drainage areas. Smaller water bodies have limited storage capacity and thus may overflow and carry pesticide in the discharge, whereas the EXAMS pond has no discharge. As watershed size increases beyond 10-hectares, it becomes increasingly unlikely that the entire watershed is planted with a single crop that is all treated simultaneously with the pesticide. Headwater streams can also have peak concentrations higher than the EXAMS pond, but they likely persist for only short periods of time and are then carried and dissipated downstream.

The Agency acknowledges that there are some unique aquatic habitats that are not accurately captured by this modeling scenario and modeling results may, therefore, under- or over-estimate exposure, depending on a number of variables. For example, aquatic-phase CRLFs may inhabit water bodies of different size and depth and/or are located adjacent to larger or smaller drainage areas than the EXAMS pond. The Agency does not currently have sufficient information regarding the hydrology of these aquatic habitats to develop a specific alternate scenario for the CRLF. As previously discussed in Section 2.5.1 and Attachment 1, CRLFs prefer habitat with perennial (present year-round) or near-perennial water and do not frequently inhabit vernal (temporary) pools because conditions in these habitats are generally not suitable (Hayes and Jennings 1988). Therefore, the EXAMS pond is assumed to be representative of exposure to aquatic-phase CRLFs.

In addition, the Services agree that the existing EXAMS pond represents the best currently available approach for estimating aquatic exposure to pesticides (USFWS/NMFS 2004).

For the analysis of defining the action area based on distances below which spray drift residues result in exposures below the LOC it was assumed when using the AgDISP model that the aerial mode was a surrogate for orchard airblast applications. The AgDISP model does not have an airblast mode for estimating drift. Therefore, there is some uncertainty with this estimation, and it is expected that the distances estimated with this tool may overestimate the total distance needed to get below the LOC; however, this is uncertain.

6.3 Terrestrial Assessment

6.3.1 Incidental Releases Associated With Use

This risk assessment was based on the assumption that the entire treatment area is subject to pesticide application at the rates specified on the label. Uneven application of the pesticide through changes in calibration of application equipment, spillage, and localized releases at specific areas of the treated field that are associated with specifics of the type of application equipment were not accounted for in this assessment.

6.3.2 Residue Levels Selection

The Agency relies on the work of Fletcher et al. (1994) for setting the assumed pesticide residues in wildlife dietary items. These residue assumptions are believed to reflect a realistic upper-bound residue estimate, although the degree to which this assumption reflects a specific percentile estimate is difficult to quantify. It is important to note that the field measurement efforts used to develop the Fletcher estimates of exposure involve highly varied sampling techniques. It is entirely possible that much of these data reflect residues averaged over entire above ground plants in the case of grass and forage sampling.

6.3.3 Dietary Intake

It was assumed that ingestion of food items in the field occurs at rates commensurate with those in the laboratory. Although the screening assessment process adjusts dry-weight estimates of food intake to reflect the increased mass in fresh-weight wildlife food intake estimates, it does not allow for gross energy differences. Direct comparison of a laboratory dietary concentration- based effects threshold to a fresh-weight pesticide residue estimate would result in an underestimation of field exposure by food consumption by a factor of 1.25 – 2.5 for most food items.

Differences in assimilative efficiency between laboratory and wild diets suggest that current screening assessment methods do not account for a potentially important aspect of food requirements. Depending upon species and dietary matrix, bird assimilation of wild diet energy ranges from 23 – 80%, and mammal's assimilation ranges from 41 – 85% (U.S. Environmental Protection Agency, 1993). If it is assumed that laboratory chow is formulated to maximize assimilative efficiency (e.g., a value of 85%), a potential for underestimation of exposure may exist by assuming that consumption of food in the wild is comparable with consumption during laboratory testing. In the screening process, exposure may be underestimated because metabolic rates are not related to food consumption.

Finally, the screening procedure does not account for situations where the feeding rate may be above or below requirements to meet free living metabolic requirements. Gorging behavior is a possibility under some specific wildlife scenarios (e.g., bird migration) where the food intake rate may be greatly increased. Kirkwood (1983) has suggested that an upper-bound limit to this behavior might be the typical intake rate multiplied by a factor of 5. In contrast, there may be potential for avoidance (animals respond to the presence of noxious chemicals in food by reducing consumption of treated dietary elements). This response is seen in nature where herbivores avoid plant secondary compounds. However, how these behaviors relate to amphibians is not clear.

T-HERPS uses avian toxicity data as a surrogate for toxicity to amphibians and reptiles. Actual toxicity data on amphibian and reptiles is frequently unavailable. Although differences in sensitivity may be expected, the lack of available toxicity data on reptiles and amphibians precludes a robust comparison to birds. This represents a source of uncertainty in the estimated risks to amphibians and reptiles. For this assessment, no terrestrial-phase amphibian toxicity data were available so birds were used as a surrogate for the terrestrial-phase CRLF.

Risk quotients calculated using the dose-based toxicity values are generally higher than RQs calculated using the dietary-based toxicity values. The dose-based approach considers the uptake and absorption kinetics of a gavage toxicity study to approximate exposure associated with uptake from a dietary matrix. Toxic response is a function of duration and intensity of exposure. For many compounds a gavage dose represents a very short-term high intensity exposure. Although the dose-based estimates may not reflect reality in that animals do not receive a gavage while feeding, it is possible that a short-duration, high-intensity exposure could occur associated with feeding on an agricultural field since many birds may gorge themselves when food items are available. Whether amphibians exhibit this type of gorging behavior is unclear. On the other hand, the dietary-based approach assumes that animals in the field are consuming food at a rate similar to that of confined laboratory animals despite the fact that energy content in food items differs between the field and the laboratory as does the energy requirements of wild and captive animals. Also, the design of dietary-based studies precludes the estimation of food consumption on a per-bird basis since birds are group housed and tend to spill feed further confounding any estimates of food consumption.

6.4 Effects Assessment

6.4.1 Estimated Effects Endpoints

The acute-to-chronic ratio method was employed in this risk assessment to estimate several chronic NOAECs for freshwater animals. There is inherent uncertainty associated with estimating toxicity endpoints since the actual endpoints may be more or less sensitive than those predicted.

6.4.2 Sublethal Effects

For an acute risk assessment, the screening risk assessment relies on the acute mortality endpoint as well as a suite of sublethal responses to the pesticide, as determined by the testing of species response to chronic exposure conditions and subsequent chronic risk assessment. Consideration of additional sublethal data in the assessment is exercised on a case-by-case basis and only after careful consideration of the nature of the sublethal effect measured and the extent and quality of available data to support establishing a plausible relationship between the measure of effect (sublethal endpoint) and the assessment endpoints.

6.4.3 Age Class and Sensitivity of Effects Thresholds

It is generally recognized that test organism age may have a significant impact on the observed sensitivity to a toxicant. The acute toxicity data for fish are collected on juvenile fish between 0.1 and 5 grams. Aquatic invertebrate acute testing is performed on recommended immature age classes (e.g., first instar for daphnids, second instar for amphipods, stoneflies, mayflies, and third instar for midges).

Testing of juveniles may overestimate toxicity at older age classes for pesticide active ingredients that act directly without metabolic transformation because younger age classes may not have the enzymatic systems associated with detoxifying xenobiotics. In so far as the available toxicity data may provide ranges of sensitivity information with respect to age class, this assessment uses the most sensitive life-stage information as measures of effect for surrogate aquatic animals, and is therefore, considered as protective of the California Red Legged Frog.

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